REPRODUCTIVE EFFORT AND SEX-SPECIFIC OFFSPRING PERFORMANCE IN THE GREAT SKUA (*CATHARACTA SKUA*)

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In Epeirus[1] the mothers used to pierce the ears of their new born sons. In that way they could cheat the Charus[2] so that he could not come and get them. He would believe that they are girls and go away.

Greek folk tale[3]

[1] Region in northern Greece
[2] Personification of death
[3] Told by Maria Alexopoulou, Athens, 2002

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Contents

Abstract

Life history theory predicts the optimisation of traits connected to reproduction in order to maximise lifetime reproductive output. Physiological and ecological trade-offs can constrain the simultaneous enhancement of two or more traits. Parents have to decide how to allocate their resources within and between reproductive events in order to maximise fitness. In a variety of circumstances differently sexed offspring can have different effects on the fitness of parents. Such a situation is likely to occur in species with sexual size dimorphism. The size difference can affect offspring performance, as has been observed in bird and mammal species with larger males. In this thesis I investigate sex-specific offspring cost and performance, reproductive costs of increased egg laying effort and parental response to these two factors in the great skua *Catharacta skua*. The great skua is a seabird with reversed sexual size dimorphism, that is the females are larger than the males. This important characteristic allows me to investigate different hypotheses that have been proposed in order to explain the vulnerability commonly exhibited by larger males, as I can separate the effect of size *per se* versus other male-specific traits. A combination of ecological field experiments and physiological measures is used to address these questions.

Female great skua chicks were found to grow to a larger final size than males and needed more total energy to reach that size, as established with the doubly labeled water method. Daughters exhibited higher mortality than sons, which was exacerbated when hatching at very low mass. Poorer hatching condition slowed down female development more than male development in chicks which fledged successfully. Hatching sex ratio of control broods was not statistically different from a 50:50 ratio. Egg removal was used to experimentally increase egg production effort. Producing additional eggs negatively affected female condition, as measured by body mass index, pectoral muscle size and haematological parameters. Egg size declined in extended sequences and mothers produced a male-biased sex ratio in later eggs. Some signs of poor female body condition could still be detected one year after the experiment. In the post-experimental year breeding was significantly delayed, but this delay was recovered two years later. Provision of supplementary food slightly affected egg composition in extended sequences, and supplemented females did not bias the sex ratio.

My results indicate that larger size is the main reason for sex-specific offspring vulnerability in size-dimorphic species, even though the disadvantage might be conferred at a time before size differences become apparent. Further, my results give strong evidence for facultative sex ratio adjustment, and the observed sex ratio is in the direction predicted for adaptive adjustment, based on higher vulnerability of daughters.

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Chapter 1

General Introduction

Successful reproduction is the ultimate aim of all organisms. Basically all traits of an organism are related to achieving this aim, but some figure more directly in reproduction than others. Some of the most prominent of these traits are size, growth, age at maturity, number and sex ratio of offspring, reproductive investment and survival (Stearns 1992). In nature we find a multitude of different combinations of these traits, from insects that spend most of their life span maturing and only have the chance to reproduce once, to some plants which can live for hundreds of years and reproduce throughout most of their life. The multitude of all these traits together is referred to as the life history of a species or individual. Like any other characteristic of an organism that affects fitness, the life history traits are thought to be subject to natural selection. How well they are adapted and what constrains them from taking on different values is a central question in the study of the evolution of life histories. Patterns of allocation are expected to be optimised for individuals. Trade-offs are a mechanism which constrains the simultaneous enhancement of two or more traits. A trade-off exists when the fitness-enhancing change of one trait negatively affects another. For example increased investment into the size of offspring might reduce the number that can be produced. Trade-offs and constraints can be observed on different levels, such as behavioural and ecological as well as physiological. In this thesis I combine the approaches of behavioural ecology and physiology to investigate the link between sex-specific cost and success of offspring, and reproductive decisions by the parents.

1.1 The cost of reproduction

The assumption that reproduction is costly is central to predictions made by life history theory. Many empirical studies give evidence for the existence of such costs. In humans, for example, raising a larger number of children appears to be connected to a decrease in life expectancy (Lycett *et al.* 2000; Mace 2000). In an analysis of a pre-industrial human population, mothers of twins were less likely to successfully raise further children compared to mothers of singletons (Lummaa 2001). Not only the number of children influenced future reproduction, but also the sex of the child: mothers of male twins were the least likely to reproduce again successfully. However, the question of what proportion different aspects of reproduction contribute to the over all costs, the physiological mechanism and the currency in which these costs are paid, have inspired a large volume of research but still remain in large parts unanswered (Roff 1992; Stearns 1992).

Strictly speaking, costs of reproduction are defined as the decrease in residual reproductive value following from investment into the current reproductive event (Sheldon & Verhulst 1996). By definition, such costs can thus only be measured during subsequent breeding events. Contrary to the predictions of reproductive costs, it has been observed that individuals which produce a high number of offspring in one season also show high future fecundity (Clutton-Brock 1991). However, such a correlation does not prove that reproduction is cost free, but result from phenotypic variation in quality between individuals. Experimental studies are important to control for such phenotypic differences. For example, experimentally increased fruit production in the pink lady's slipper orchid *Cypripedium acaule* resulted in a decreased flowering probability and smaller leaves in the following season (Primack & Stacey 1998). In the collared flycatcher *Ficedula albicollis* caring for an experimentally increased number of chicks one year reduced future fecundity of females (Gustafsson & Sutherland 1988). However, in practical terms, biologists across all fields have used the term 'costs of reproduction' also for any immediate negative effects of reproduction on any features that are known to affect fitness (Clutton-Brock 1991). In indeterminate growers, such as fish or plants, there is an immediate trade-off between energy channelled towards reproduction and energy invested into growth, where larger size is related to higher reproductive success (Vanrooij *et al.* 1995; Fleming 1996; Mendez 1999). In many animals a reduction in body condition is observed during reproduction, which can vary with the sex of offspring that is being raised (Festa-Bianchet 1989) and often worsens when investment is increased (Dijkstra *et al.* 1990; Golet & Irons 1999; Murphy *et al.* 2000). Reproductive costs can thus be divided into immediate (paid during the same reproductive event) and delayed costs (paid during subsequent reproductive events). The common phenomenon of reduced body condition due to reproductive activities is thought to form a link between current investment and reduced future reproduction (Roff 1992; Stearns 1992; Sheldon & Verhulst 1996).

Amongst the higher vertebrates, birds have widely been used to test predictions of life history theory about costs and constraints of reproduction. Lack's (1947) suggestion that clutch sizes are ultimately limited by the number of offspring parents can raise successfully, set the scene for many empirical studies on causes of clutch size restriction. Despite a large body of work devoted to this question, the debate about to what extent different phases of reproduction restrict the number of fledglings produced and via which mechanism is still ongoing. Several studies which manipulated brood sizes found that the brood size most often observed in nature (modal brood size) was not the one that produced most fledglings (optimal brood size) (VanderWerf 1996). Despite the knowledge that egg

production represents a considerable portion of a female's energy expenditure (Robbins 1983; Carey 1996), the impact of producing and incubating eggs was mostly considered negligible in terms of constraining the number of eggs laid (Stearns 1992; Monaghan & Nager 1997). However, models which include the costs involved in the processes leading up to a given brood size, such as egg production and incubation, might better explain the clutch sizes observed in nature. So far, only very few studies have looked at the costs of egg production alone, with incubation and chick rearing being kept at the same level as in a control group. The results of these studies show that increased investment into egg production can decrease offspring rearing capacity in the same year (Heaney & Monaghan 1995; Monaghan *et al.* 1998), as well as future reproductive output and survival (Nager *et al.* 2001; Visser & Lessells 2001). As the female is expected to bear most of the immediate costs of egg production, studies are now increasingly focussing on the effect of egg production on female body condition as a link to the indirect reproductive costs. So far physiological trade-offs have been found with parasite resistance (Oppliger *et al.* 1996), protein condition (Houston *et al.* 1995) and flight performance, leading to increased predation risk (Veasey *et al.* 2001). Results like these are starting to change the view on egg production and its possible impact on clutch size limitation. More studies are needed to establish a better knowledge of the immediate costs of egg production and link them to reproductive output and survival of females as well as of their partners.

1.2 Sex-specific offspring vulnerability

As mentioned above, offspring sex can directly influence the immediate costs of reproduction to parents. Equally, the sex of offspring can indirectly affect residual reproductive value of parents. If, all else being equal, one sex has the potential to sire more offspring of its own than the other sex, then that sex will add more to the residual reproductive value of the parents. It has indeed been found that in several species the sexes differ in their life histories and expected reproductive success under the same conditions (Lindström & Kokko 1998 and references therein). Such a situation is easy to imagine in polygynous species, that is that one male is paired with several females simultaneously. Differences in reproductive value between the sexes are less obvious in monogamous species with biparental care. In humans for example, one would expect on average the same number of grandchildren from a son as from a daughter. However, this assumption could change if one sex is less likely to reach maturity. Such a difference in offspring vulnerability has not only been found in humans (Elo & Preston 1992; Stevenson *et al.* 2000), but also in other vertebrates (Lindström 1999). As in humans, in many other

mammals and bird species males tend to exhibit higher vulnerability as foetuses, neonates or during the period of parental care (Howe 1977; Clutton-Brock *et al.* 1985; Griffiths 1992; Desportes *et al.* 1994; Monard *et al.* 1997). Mainly with mammals in mind, biologists have put forward different hypotheses to explain this pattern. Particularly three hypotheses have been explored and discussed widely. The size-hypothesis suggests that due to their larger average size, males have a higher energy demand during growth, which in turn makes them more vulnerable to starvation (Clutton-Brock *et al.* 1985; Weatherhead & Teather 1991). The heterogameity hypothesis postulates that the possession of the two different sex-chromosomes (XY) makes males more vulnerable to the effects of deleterious mutations on the X chromosome (Myers 1978; Smith 1989). Female cells possess a second X chromosome which is likely to provide a non-mutated copy of the DNA region in question. By contrast, the Y chromosome only carries a minor fraction of the genes found on the X chromosome, and will therefore not be able to mask the negative effects of a deleterious mutation in a coding region of the X chromosome. Lastly, the immunocompetence handicap hypothesis (ICHH) is based on the assumption of a negative effect of testosterone on immune function (Folstad & Karter 1992). Since males experience higher levels of circulating testosterone during gonadal development, they might incur a decreased ability to fight disease or parasites. Even though originally stated specifically for testosterone, the scope of the ICHH has been widened by some authors to include any biologically active substances that are particular to the male phenotype and negatively affect other aspects of physiology (Sheldon *et al.* 1998).

Empirical evidence from studies of mammals and birds is equivocal so far for all three hypotheses. While the negative effect of testosterone on immune function is sometimes taken to be fact, some recent experimental studies suggest that this effect is not straightforward. In an experiment with greenfinches *Carduelis chloris* Lindström *et al.* (2001) showed that higher testosterone levels did not increase viraemia as such, but altered the course of the infection. Equally, Evans *et al.* (2000) found that testosterone did not decrease antibody production in male house sparrows *Passer domesticus*, but rather increased it. Only through the interaction with corticosterone did it affect antibody production. But Nolan *et al.* (1998) interpreted the male-biased mortality during a virus infection in house finches *Carpodacus mexicanus* as support for the ICHH.

The impact of heterogameity on vulnerability is more complicated to investigate and can usually only be inferred indirectly. However, one indication about the relative importance of heterogameity comes from the comparison of birds and mammals. Contrary to mammals, in birds the females carry two different sex chromosomes (Mittwoch 1996).

The finding that not only in mammals, but also in birds male offspring often exhibit higher mortality (Clutton-Brock *et al.* 1985; Griffiths 1992) suggests that the relative contribution of heterogameity is small. However, a study which manipulated rearing conditions in the monomorphic zebra finch *Taeniopygia guttata* found long term effects on female survival that were interpreted as support for the heterogameity hypothesis (De Kogel 1997).

The size hypothesis is often unquestioningly believed to be true. However, the majority of evidence comes from observational studies, while support from experimental studies is very limited. Most studies suffer from the problem that males are the larger offspring sex, and it is therefore impossible to distinguish between the effects of size and other aspects of male phenotype on vulnerability. In particular species where females are larger, also referred to as species with reversed sexual size dimorphism (RSD), may provide a better insight into the relationship between offspring size and vulnerability. RSD is very rare in mammals, but is found in a number of birds, most prominently in the group of raptors (del Hoyo *et al.* 1994). So far, there is only very limited evidence from species with RSD. While two studies have shown higher energy requirements of female marsh harrier *Circus aeruginosus* nestlings, (Krijgsveld *et al.* 1998; Riedstra *et al.* 1998), only one observational study has reported higher female mortality when females are the larger sex (Torres & Drummond 1999). In an experimental study of a monomorphic species the absence of sex-specific vulnerability was interpreted as support for the size hypothesis, as both other mechanisms (ICHH and heterogameity) would also act when the sexes are the same size (Sheldon *et al.* 1998).

Lastly, it is important to keep in mind secondary effects that might counteract the effects of the above mentioned primary causes of vulnerability. In particular inter-offspring competition might play an important role. In many cases larger offspring are likely to have a competitive advantage over smaller ones (Mock & Parker 1997). This can give them priority access to food. In times of food scarcity this can divide the burden unevenly between larger and smaller offspring, in that larger offspring suffer decreased nutritional pressure, because they can secure better access to food, while small offspring suffer increased pressure. A competitive advantage of larger individuals is for example thought to cause the sex-specific mortality pattern in great tits *Parus major*, where the smaller females appear to suffer more in unfavourable circumstances (Oddie 2000).

1.3 Sex allocation

At the beginning of this introduction I outlined briefly that natural selection is expected to optimise life history traits in order to achieve maximum reproductive success

for individuals. Sex-specific vulnerability can influence the reproductive value of male and female offspring. There are many other situations in which one sex is expected to provide a greater fitness return to the parents than the other (Charnov 1982). Such a situation can for example exist when there are differences in dispersal or helping behaviour between the sexes (Silk 1983; Emlen *et al.* 1986; Gowaty 1993). In some species the attractiveness of the father might confer larger fitness advantages to sons than to daughters (Burley 1981; Ellegren *et al.* 1996), while in others the value of offspring might vary with the condition or social rank of the mother (Cameron *et al.* 1999; Côté & Festa-Bianchet 2001). Given that under certain circumstances male and female offspring can be of different reproductive value to parents, we would predict that parents have evolved the capacity to allocate their resources to the two sexes accordingly. This allocation can take place at different levels. Once the offspring are born, parents can preferentially care for or provision members of one sex (Nishiumi *et al.* 1996; Koenig *et al.* 2001). Similarly, parents might increase their effort towards broods that contain a high ratio of the wanted sex (Radford & Blakey 2000). Both scenarios mainly refer to species with brood sizes of more than one. Also, these strategies of differential allocation appear to be an energetically expensive way to adjust investment, as parents already invest resources into offspring prior to birth. A preferable strategy would be to adjust the primary offspring sex ratio. Following their studies on red deer *Cervus elaphus*, Trivers and Willard (1973) suggested that parents should have evolved the ability to influence the primary sex ratio when offspring value differs with maternal condition. Due to the polygynous mating system in red deer, the expected reproductive output of male calves benefits to a higher degree from good early rearing conditions than the reproductive value of female calves (Kruuk *et al.* 1999). Thus, mothers which are in a physical state to provide good conditions during foetal and post-natal growth should produce more sons, as mothers can expect a higher fitness return from sons for the same increase of investment than they would get from daughters. Trivers and Willard's hypothesis was later extended to include any situation in which sex-specific fitness returns vary (Charnov 1982). While such adaptive manipulations of the primary sex ratio have for example been found in some insects, such as parasitoid and fig wasps (Godfray 1994), empirical evidence from mammals and birds has been scarce (Clutton-Brock & Iason 1986; Gowaty 1991; Hewison & Gaillard 1999). Some deviations from a 50:50 ratio have been observed and have intrigued many researchers, such as an apparently adaptive example from a human population. During and after World War I and II the number of male births peaked in many countries (Vartiainen *et al.* 1999; Jongbloet *et al.* 2001). An increase in male births would be consistent with adaptive sex ratio adjustment,

as fitness returns from boys could be expected to be larger than from girls, due to the loss of young men in the war.

The ability of higher vertebrates to produce sex ratio skews was thought to be constrained by the chromosomal sex determining mechanism of birds and mammals (Hardy 1997). According to Mendelian laws of inheritance, this system should produce a 50:50 ratio of sons and daughters. In birds, which have been used as model organisms to test many life history predictions, the widespread study of primary sex ratios was for a long time hampered by the difficulty of sexing newly hatched chicks or even embryos. Following the development of relatively cheap, easy and almost universally applicable DNA based methods to sex birds (Ellegren 1996; Griffiths *et al.* 1996; Griffiths *et al.* 1998), the study of primary sex ratios in birds has intensified again. Some recent studies have shown very interesting results and given strong support for the existence of a parental capacity to adaptively adjust the primary offspring sex ratio (Appleby *et al.* 1997; Komdeur *et al.* 1997; Nager *et al.* 1999; Sheldon *et al.* 1999). Whether the ability to adjust the primary sex ratio is common to all birds, whether the results of those studies are some spurious results, or whether primary sex ratio biases are simply a physiological by-product and only by chance agree with prediction of adaptiveness presently remains a topic of vivid discussion (Gowaty 1991; Krackow 1995; Emlen 1997; Sheldon 1998).

1.4 The great skua

The great skua *Catharacta skua* belongs to the group of Stercorariinae (skuas), a subfamily of the Laridae, within which they are closely related to the Larinae (gulls) (Sibley & Ahlquist 1990; Olsen & Larsson 1997). The skuas possess some typical features that distinguish them from the gulls, mainly connected to the more predatory lifestyle of the former (Furness 1987). One of these features is reversed sexual size dimorphism, exhibited by all members of this subfamily (Catry *et al.* 1999). Like most seabirds, great skuas breed in colonies, even though they defend territories (Furness 1987). These characteristics together make them an ideal species to study sex-specific offspring vulnerability and its effects on parents' reproductive decisions. The aspect of colony nesting is helpful in a practical way, as it facilitates access to a large number of breeding pairs and thereby creates the possibility to carry out experimental work. As pointed out above, reversed sexual size dimorphism allows the researcher to tease apart effects of size and 'male-ness', and therefore adds a crucial dimension to the investigation of sex and size related life history questions. Finally, the close relatedness to gulls provides an opportunity for direct comparison, as most gulls exhibit male-biased size dimorphism. This is also the

case in the lesser black-backed gull *Larus fuscus,* for which a number of studies about offspring vulnerability, sex allocation, effects of offspring sex on parental fitness and parental condition have been published in recent years (Griffiths 1992; Bolton *et al.* 1993; Monaghan *et al.* 1998; Nager *et al.* 1999; Nager *et al.* 2000; Nager *et al.* 2001).

The great skua population where I carried out the work for my study breeds on the island of Foula, Shetland, and has been the subject of a long-term study for more than 30 years. On the whole, the population supports more than 2000 breeding pairs, and in the study area several hundred birds are ringed with individual colour-combinations (Ratcliffe *et al.* 2002). The ongoing study has provided relatively detailed knowledge about several life history aspects. Great skuas are long-lived and can reach more than 30 years of age. Adult survival is generally high and lies between 85 and 95 % (Ratcliffe *et al.* 2002). Pairs tend to breed together for many years, with low rates of divorce and usually occupy the same territory year after year (Catry *et al.* 1997). Clutch sizes are never larger than two, but if eggs or clutches are lost skuas can produce replacement eggs (Furness 1987). Egg size influences the growth of chicks, and hatchling size is closely related to egg size (Furness 1983). Laying date is regarded as a good indicator of parental quality in this species (Catry *et al.* 1998), while breeding success improves with age, as is the case in most seabirds (Ratcliffe *et al.* 1998). However, as skuas do not exhibit plumage dimorphism, sexing of young birds is only possible via DNA, and of adult birds through observation of courtship feeding or copulation behaviour (Catry & Furness 1997), and to a certain extent from morphometric measurements (Hamer & Furness 1991). So far, nothing is known about nestling sex ratios or differences between the sexes in chick performance in the great skua, and only one study has reported the nestling sex ratio of another skua species, the brown skua *Catharacta lonnbergi*, where no sex-bias was found (Millar *et al.* 1992).

1.5 Outline of thesis

In **chapter 2** I use an experimental approach to test if female great skuas skew the primary sex ratio in relation to their body condition. Such a skew has been observed in eggs of the lesser black-backed gull, where mothers overproduced daughters, the smaller and less vulnerable sex when in poor body condition. In the experimental protocol I use egg removal as a way to influence maternal condition. The connection between increased egg production and deteriorating female condition has been shown in lesser black-backed gulls, and in **chapter 3** I investigate the extent to which extra egg production affects female condition in the great skua. As increased investment into egg production is likely to

incur delayed costs of reproduction, I also follow the recovery of body condition of experimental females in the following year.

Another aspect of the experimental protocol used for the experiment in chapter 2 is supplementary feeding as a means to counteract the effects of egg laying on body condition and enhance egg laying capacity of females. Supplementary feeding is a method widely used in life history studies, and in **chapter 4** I investigate the effects it had on egg composition, compared to eggs of un-fed females.

The following three chapters concentrate on differences in chick performance between the sexes. In **chapter 5** I investigate differences in mortality of male and female chicks. As well as following un-manipulated broods, I employ an egg removal and cross-fostering protocol to separate effects of egg quality, parental condition and sibling interaction on chick survival. Several aspects of growth of male and female chicks are examined in **chapter 6.** Besides following mass gain, I also present data on wing and tarsus growth, the relative timing of these growth aspects in males and females and the effect of hatching condition on growth. In **chapter 7** I use doubly labeled water to measure daily energy expenditure of free-living chicks and present results of carcass analysis with respect to organ sizes.

In **chapter 8** I return to the parents' perspective and investigate inter-seasonal costs of increased investment into egg production. **Chapter 9** represents a brief synthesis of the findings of the previous chapters, and I outline questions arising from my work and their implications for future work in this field.

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Chapter 2

Increased reproductive effort results in male-biased offspring sex ratio: an experimental study in a species with reversed sexual size dimorphism

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2.1 Abstract

Adaptive sex ratio theory predicts that parents should overproduce the more beneficial offspring sex. Based on a recent experimental study of lesser black-backed gulls, we tested this hypothesis with the great skua, *Catharacta skua*, a bird species closely related to gulls but where females are the larger sex. When in poor body condition, the gulls overproduced daughters, the smaller and more viable sex under those circumstances. To discriminate between a mandatory physiological overproduction of female (i.e. non-male) eggs versus the overproduction of the smaller and presumably more viable sex, we conducted an egg removal experiment with the great skua. Since the males are smaller, larger size and being male are separated. Through egg removal we induced females to increase egg production effort. Eggs were sexed using a DNA based technique. Manipulated pairs produced a significant male bias at the end of the extended laying sequence, while the sex ratio in the control group did not differ from unity. Our results present an example of facultative sex ratio manipulation and support the hypothesis that in sexually dimorphic birds parents overproduce the smaller sex under adverse conditions.

2.2 Introduction

In recent years, several studies have given strong evidence that birds can adjust the sex ratio of their offspring in relation to environmental conditions (Ellegren *et al.* 1996; Appleby *et al.* 1997; Komdeur *et al.* 1997; Nager *et al.* 1999; Sheldon *et al.* 1999). The theory behind sex ratio adjustment hinges on the observation that an environmental variable can have different effects on the reproductive value of male and female offspring, and hence the sex ratio bias is expected to be adaptive (Trivers & Willard 1973; Sheldon *et al.* 1998). Such environmental effects on the offsprings' reproductive value have been observed in a number of species, for example in relation to territory quality (seychelles warbler, *Acrocephalus sechellensis,* Komdeur *et al.* 1997), paternal attractiveness (collared flycatcher, *Ficedula albicollis*, Ellegren *et al.* 1996), or clan size in mammals (spotted hyena, *Crocuta crocuta*, Holekamp & Smale 1995). A common factor across a wide range of species is differential sensitivity to adverse conditions, which often results in sex-biased mortality (Røskaft & Slagsvold 1985; Clutton-Brock *et al.* 1985; Griffiths 1992). In birds, males generally show higher mortality than females (Howe 1977; Røskaft & Slagsvold 1985; Breitwisch 1989; Griffiths 1992; Nolan *et al.* 1998). Since males are typically larger than females, this has led to the hypothesis that males are more vulnerable to adverse conditions due to their relative size and the size-linked higher energy demand (Clutton-Brock *et al.* 1985). Studies on species where males are not the larger sex support this hypothesis. In an experimental study on a monomorphic species, no sex-linked differences in performance could be detected (Sheldon *et al.* 1998), and observational data on a bird with reversed sexual size dimorphism reported female-biased nestling mortality (Torres & Drummond 1997).

A recent study on lesser black-backed gulls, *Larus fuscus*, a species with male-biased size dimorphism, has given experimental evidence for a link between poor maternal condition, male-biased nestling mortality, and a female-biased primary sex ratio (Nager *et al.* 1999). Mothers in poor body condition overproduced daughters, which had a higher survival probability than sons under those circumstances. Even though this experiment suggests a directional overproduction of daughters due to their smaller size, the possibility of physiological constraints causing a skew towards daughters under food stress can not be excluded. Conducting an experiment similar to the gulls in a species where females are the larger sex would be a strong exploration of the mechanistic versus the facultative sex ratio adjustment hypothesis. If mothers in poor condition show a mandatory overproduction of female eggs, then we also expect to find this pattern in a species where the females are larger than males. However, if the gulls produced more daughters because they are the

smaller and less vulnerable sex, then we would predict a similar overproduction of the smaller sex, in this case the males.

In order to distinguish between these hypotheses, we performed an egg removal experiment with great skuas, *Catharacta skua*. Great skuas are closely related to gulls (Cohen *et al.* 1997). Unlike the gulls, however, they show reversed sexual size dimorphism, with females being on average 10% larger than males (Furness 1987). Through continuous egg removal we induced mothers to lay extended clutches and thus increased the amount of energy invested into egg production. Great skuas normally lay clutches of two eggs (Furness 1987), but produced on average three times as many eggs due to our manipulation. This elevated investment negatively affects maternal condition (Monaghan *et al.* 1998; Veasey *et al.* 2001), and female gulls in poorer body condition produced less viable offspring (Nager *et al.* 2000), and showed a lower offspring rearing capacity (Monaghan *et al.* 1998). In order to counteract the effects of egg laying on maternal condition, we supplied a second group of females whose eggs were continuously removed with supplementary food during egg formation. We investigate the primary sex ratio of eggs laid by females with declining body condition and that produced by the fed group. If birds manipulate offspring sex ratio in relation to the size of their offspring, rather than always favouring daughters under poor conditions, we predict an increase of proportions of sons as egg production effort increases in the un-fed skuas. No sex ration bias is expected for the fed birds, since their condition is unlikely to decline by a large degree through the experiment.

2.3 Methods

The study was carried out at a large colony of great skuas in Foula, Shetland (60°08'N,2°05'W), during May and June 1999. We used 70 pairs of great skuas for our experiment and carried out daily nest checks prior to and during egg laying. 92 of our study birds were already ringed with unique colour combinations, and the other 48 could mostly be identified through individual plumage markings. From these nests every new egg was removed within 48 hours of laying, which induced the birds to lay further eggs. The minimum observed time span needed by great skuas to produce replacement eggs after natural clutch loss is ten days (Steve Votier and Stuart Bearhop, pers. com.). We therefore report the number of females with or without inter-egg intervals of 10 or more days. Egg removal continued until birds stopped relaying. Since study pairs remained on their territories for several weeks after the removal of the last egg, we could be sure that they had not changed partner or territory during or directly after the experiment. 38 randomly

assigned study pairs received daily portions of supplementary food from at least 10 days prior to laying of the first egg. We fed them with 400g of tinned cat food per pair per day, which contained an average of 1100 kJ, equivalent to approximately 30% of the daily energy need of one pair (Ratcliffe 1993). Since the males undertake most of the foraging and then provide food for the female (Furness 1987), it did not matter which of the two partners was present at the time of feeding. In order to avoid the theft of food by non-experimental birds, feeding took place in the late evening, a time with generally high territorial attendance. All pairs readily accepted the food and, due to the individual markings, we were sure that only target birds took the food. One of our original pairs in the un-fed group abandoned the breeding attempt after the removal of the first egg, but stayed and defended their territory during the remaining breeding season. This pair was not included in any of the following analyses.

Egg length and breadth was measured and egg volume index in mm^3 was computed by $breadth^2$ x length. Removed eggs were artificially incubated for approximately five days. The complete natural incubation period is 29 days. On day five eggs were opened, the embryos removed, stored in a buffer containing 50 mM EDTA, 50 mM Tris and 2% SDS, and frozen at –20°C. For sexing we used the method described by Griffiths et al. (1998), but used primers PF31 (TGA AGT ATC GCC AGT TYCC) and PR32 (CTT CCT CAA TTC CCC TTT DAT), at an annealing temperature of 55°C. Sexing of eggs that had not developed a visible embryo was inconsistent, and it is not clear whether the obtained results are reliable (for example possible contamination by sperm). Therefore, those eggs were not included in the analysis. Sex ratios are given as proportion of males.

Except for one pair in the fed group, which only laid two infertile eggs, a final clutch size of three was the minimum produced by all pairs. This gave us a final sample size of 37 fed pairs and 31 un-fed pairs for the sex ratio analysis. To prevent bias towards pairs which had laid a high number of eggs, we included three eggs of all pairs in the analysis: the first, middle and last egg of the laying sequence. Middle eggs in sequences with an even egg number were selected following a table of random numbers. By including eggs of the same relative position within each laying sequence, we aim to use eggs of a similar biological significance for each female. Any eggs laid between these three positions are referred to as intermediate eggs: intermed1 between first and middle, and intermed2 between middle and last egg. In some cases, particularly later eggs were inadvertently left with the parents, and were therefore not available for analysis. Whenever the sex of the first or last egg was not known, we used the sex of the egg next to it in the laying sequence, provided it was still laid during the same third of the clutch (see also

Nager *et al.* 1999). We had to substitute 2 of 68 first eggs and 10 of 46 last eggs (6 un-fed and 4 fed pairs). The percentage of eggs for which the sex could not be determined due to the lack of a visible embryo was not different between the fed and the un-fed group (fed: 84.4% sexed, $n = 224$; un-fed: 87.2% sexed, $n = 179$; $G_1 = 0.621$, p = 0.43). To analyse sex ratios and egg viability between treatment groups we fitted a general linear mixed model (GLMM) with a binomial error structure (Schall 1991; Kruuk *et al.* 1999), using the Genstat 5 package, version 4.1. As fixed factors we initially included treatment, egg position (first, middle, last) and laying date, plus all the ensuing two-way interactions, but only statistically significant interactions are reported. We incorporated female identity as a random factor, in order to control for individual effects, thereby accounting for the problem of pseudoreplication. It is quite common with empirical data that the residual variation of the fitted logistic regression model deviates from the binomial sampling variation assumed by the model (Collett 1991; Williams 1982). This is what we also observed during analysing the current data set. Therefore, we included estimation of the dispersion parameter in the GLMM fitting. The significance of the explanatory variables was determined by their Wald statistics (χ^2 distributed). All other statistical analyses were performed using SPSS for Windows, version 9.0. To calculate sex ratios of intermediate eggs, females contributed a maximum of one data point for intermed1 and intermed2, using the combined sex ratio, if several eggs per female fell into the respective category. Data are presented as means ± s.e. with *n* denoting sample size. All statistical tests are two-tailed and the significance level was set at 5%.

2.4 Results

Birds in the two experimental groups, i.e. fed and un-fed, both laid a mean of around six eggs over the season (fed: 6.6 ± 0.3 eggs, $n = 38$; un-fed: 6.0 ± 0.3 eggs, $n = 31$; $t_{67} = 1.4$, $p = 0.17$). For a number of pairs no last egg was available (see methods), but this does not bias the analysed sample towards larger (number of eggs laid by pairs not included in last egg analysis: un-fed: 6.3 ± 0.47, $n = 7$; fed: 6.1 ± 0.44, $n = 15$, number of eggs laid by pairs contributing to analysis: 6.4 ± 0.26, $n = 46$). The minimum number of eggs laid was two, the maximum ten. Inter-egg intervals between consecutive eggs ranged from 2 to 20 days, but were not different between treatment groups (un-fed: 5.2 ± 0.42 days, $n = 28$; fed: 5.0 ± 0.17 days, $n = 37$; $t_{63} = 0.72$, $p = 0.47$). Likewise, the occurrence of laying intervals of 10+ days was equal in the fed and un-fed group (0 / 1 / 2 10+ intervals: un-fed: 13 / 14 / 4; fed: 14 / 18 / 5; $\chi^2 = 0.12$, df = 2, p = 0.94). Timing of laying was also

equal for the two groups, the initiation of clutches (median Julian laying date of first egg for fed = 135, $n = 38$; un-fed = 134.5, $n = 31$; Mann-Whitney U-test $z = 0.50$, $p = 0.62$), as well as the date when last eggs appeared (median Julian laying date of last egg for fed = 193, $n = 22$; un-fed = 191.5, $n = 24$; Mann-Whitney U-test $z = 0.99$, $p = 0.32$). Most experimental birds were not of known age, but they had all been observed as breeding birds in previous years. There is no reason to assume an age difference between the two groups. Fed birds did not lay bigger eggs than un-fed birds (volume index for first eggs only, fed: 175.5 ± 2.3 mm^3, $n = 38$; un-fed: 172.4 ± 3.2 mm^3, $n = 31$; $t_{67} = 0.79$, $p = 0.43$) and there was no relation between egg size and embryo sex (GLMM, sex as a function of egg volume, pair identity included as random factor: Wald statistic (χ^2) = 0.5, df = 1, $p = 0.48$, $n = 174$).

Table 2.1: Results of a GLMM with a binomial error structure. Sex was examined as a function of egg position (first, middle or last egg of extended clutch) and parental feeding treatment (fed or un-fed, $n = 174$ eggs). The initially included explanatory variable 'laying date' did not contribute significantly to the model (Wald statistic(χ^2) = 0.1, df = 1, $p = 0.75$). Breeding pair identity was included as a random factor (average effect 0.7149 ± 0.44) and the data was scaled according to a dispersion parameter of 0.8795 ± 0.12.

	average effect	**s.e.**	**Wald statistic (χ^2)**	**d.f.**	***p***
Position	-0.2186	0.1926	1.3	1	0.25
Feeding treatment	0.3245	0.3667	0.7	1	0.40
Position * feeding treatment	0.8510	0.3864	4.8	1	0.03

The sex ratio of first and middle eggs was not different from unity for either of the two groups (sex ratio first eggs, fed: 0.54 ± 0.08, $n = 37$, un-fed: 0.45 ± 0.09, $n = 31$; middle eggs, fed: 0.52 ± 0.09, $n = 31$, un-fed: 0.62 ± 0.09, $n = 29$, fig. 2.1). This was also the case for the last eggs of fed birds (0.45 ± 0.11, $n = 22$, fig. 2.1). However, the un-fed birds showed a significant overproduction of males in the last eggs (0.75 ± 0.09, $n = 24$; fig. 2.1). The interaction term of position and feeding treatment in the GLMM is significant (tab. 2.1), which means the change in sex ratio from the first to the last egg

differs in the two treatment groups. Even though the sex ratio of middle eggs from un-fed birds is not different from a 0.5 binomial distribution, the sex ratio over the three egg positions in un-fed birds was progressively skewed towards an overproduction of males (GLMM, sex as a function of egg position, un-fed pairs only, pair identity included as random factor: Wald statistic (χ^2) = 5.7, df = 1, p = 0.02, n = 85; effect of position: -0.6747 ± 0.28). By contrast, fed birds produced equal numbers of male and female eggs at the beginning, middle and end of the laying sequence (GLMM, sex as a function of egg position, fed pairs only, pair identity included as random factor: Wald statistic (χ^2) = 0.4, df = 1, p = 0.53, n = 89). Laying date, as a potential measure of parental quality, did not have a significant impact on the sex ratio produced by either group (tab. 2.1). The probability to find an embryo did not differ between treatments and was unrelated to position (GLMM, presence of embryo as a function of egg position (χ^2 = 2.2, df = 1, p = 0.14); treatment (χ^2 = 0.1, df = 1, p = 0.75); position*treatment (χ^2 = 1.9, df = 1, p = 0.17); pair identity was included as random factor).

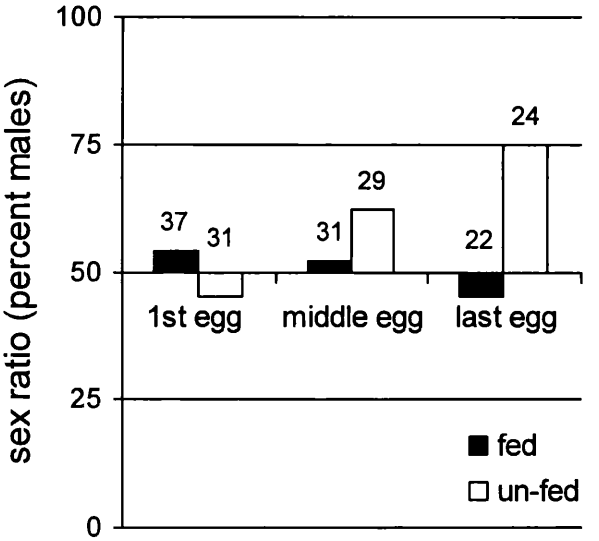

Figure 2.1: Mean sex ratio of first, middle and last eggs of fed and un-fed birds. Sex ratios for fed pairs in all three positions is not different from a 0.5 binomial distribution (binomial test, exact values; $p > 0.7$ for all three). Sex ratio of last (p = 0.023), but not of middle or first eggs ($p > 0.2$ for both) of un-fed pairs is significantly different from a 0.5 binomial distribution, and also from the sex ratio of the fed pairs' last eggs (χ^2 = 4.2, df = 1, p = 0.04). The interaction of feeding treatment and position is significant (see tab. 2.1). Sex ratios of intermediate eggs are: un-fed intermed1 0.42 ± 0.08, n = 26 females, 43 eggs; intermed2 0.51 ± 0.11, n = 18 females, 28 eggs; fed intermed1 0.44 ± 0.07, n = 34 females 59 eggs, intermed2 0.44 ± 0.09, n = 25 females, 38 eggs.

2.5 Discussion

Female great skuas produced an offspring sex ratio in favour of sons, the smaller sex, when they were induced to increase egg production effort. Birds which were subjected to the same experimental manipulations but received supplementary food, did not produce a skewed sex ratio. These results are strong evidence for facultative sex ratio adjustment in relation to maternal body condition. The fact that mothers who received supplementary food did not produce a sex ratio different from unity demonstrates that the result in the un-fed group was neither a seasonal nor a sequence effect. Since our two experimental groups did not differ in any aspect other than the receipt of supplementary food, it is clear that the overproduction of male eggs by the un-fed birds was induced by the effects of continuous laying under natural feeding conditions. Our results show that the change in offspring sex ratio is not simply produced by a physiological process, which favours female eggs when mothers are in poor body condition. The skew we observed is opposite to the one produced by lesser black-backed gulls with regard to offspring sex, but in the same direction with regard to size dimorphism (Nager *et al.* 1999). The gulls were subjected to a similar treatment, but overproduced daughters (the smaller sex) at the end of the extended laying sequence when not supplementary fed. Our results therefore clearly show a sex-independent overproduction of the smaller offspring sex by mothers in poor body condition, and are consistent with the predictions of the facultative sex ratio adjustment hypothesis. An alternative explanation for the skewed sex ratio in 5 day old eggs might be differential early embryo mortality. This possibility cannot be excluded, but is unlikely. The probability to find an embryo was the same in both groups, and we therefore have no reason to assume that differential mortality would have operated only in one of the groups. Furthermore, no differences in embryo mortality between sexes have been reported for birds so far, even in species that show differential nestling mortality (e.g. Nager *et al.* 1999). We therefore suggest that differential production is the more likely explanation for the observed sex ratio bias.

In both studies, Nager *et al.*'s (1999) and our own, poor conditions were created by inducing females to produce extra eggs. It has been shown that egg production is costly for female birds (Houston *et al.* 1983; Monaghan & Nager 1997; Veasey *et al.* 2001). Even the production of one or a few additional eggs significantly reduced protein condition of laying females (Monaghan *et al.* 1998; Veasey *et al.* 2000). Our experimental females produced on average three times more eggs than the normal clutch of two. Extended egg production had a negative effect on skua body condition (chapter 3). Even though we cannot quantify the extent to which the supplementary food counteracted these negative effects, other

studies have shown that supplementary fed birds laid larger and more eggs (Bolton *et al.* 1993; Nager *et al.* 1999), both usually correlated with body condition (e.g. Martin 1987). A trend in the same direction, with more eggs laid by supplemented skuas, was observed in this study. Therefore, it is likely that at the end of laying maternal body condition was poorer in the un-fed group and was the proximate cause for the change in offspring sex ratio.

Individual quality of females is likely to have varied within each treatment group. High quality birds were probably less affected by our treatment than lower quality individuals in the same group. We tried to control for this variation by including laying date in our model, which in this species has been shown to correlate with several reproductive traits (Catry *et al.* 1998). We could not find a significant effect of laying date on sex ratio, but it is likely that intra-group differences in individual female quality are contributing to the extra binomial variance we found in out data. Since harsh circumstances highlight such differences in quality, performance in the stressed, i.e. un-fed group, is likely to have varied to a higher degree than within the fed group.

The fact that both gulls and skuas overproduced the smaller offspring, irrespective of sex, strongly indicates that larger size per se affects offspring value under poor conditions. This is particularly interesting since other mechanisms have been suggested for sex-specific offspring performance. For instance, Folstad and Karter (1992) formulated the immunocompetence handicap hypothesis (ICHH), which states that males are more sensitive to adverse conditions due to elevated levels of androgens. Even though usually argued to relate to testosterone, the above argument would equally apply to any biologically active substances which are linked to the male phenotype and have negative effects on physiological functions (Grossman 1985; Folstad & Karter 1992). However, since males are often the larger sex in birds and mammals, it is difficult to separate size from other aspects of the male phenotype, in order to test the ICHH hypothesis. The indications of our study are that hormonal effects of male phenotype on vulnerability may be small in comparison to the effects of size.

What could be the benefits for parents to produce predominantly the smaller sex during adverse conditions? Generally, it is thought that the smaller sex needs less energy in order to achieve normal growth (Lindén 1981; Slagsvold *et al.* 1986; Krijgsveld *et al.* 1998). This would mean that by producing a cheaper chick, parents could allocate more energy to body reserves. Likewise, with the same amount of energy they could produce a higher quality fledgling, or, alternatively, may only be able to successfully raise chicks of the cheaper sex, due to limited resources (Nager *et al.* 2000). However, Torres and

Drummond (1999) concluded from their study on blue-footed boobies, *Sula nebouxii*, that despite their larger size, daughters did not need more energy to achieve normal growth. This suggests that there might be factors involved other than energy demand during the nestling phase. An important aspect for sex-specific viability may lie within the eggs themselves. In the lesser black-backed gull the survival probability of sons (the larger sex) hatching from late eggs of an extended laying sequence was significantly decreased compared to their sisters', after controlling for parental condition (Nager *et al.* 1999). In natural conditions both, parental condition and egg quality, would operate at the same time.

Species with reversed sexual size dimorphism offer an important opportunity in investigating the role of size and other aspects of phenotype on offspring vulnerability. Our results on the great skua suggest that birds might be able to adjust the primary sex ratio accordingly. They also indicate that size plays possibly the most important role for differential performance of offspring. If female mortality in our study species is shown to be higher than male mortality under poor conditions, this could explain the pattern of offspring sex ratio observed in this study.

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Chapter 3

Short- and long-term effects of increased egg production on female body condition

Manuscript:

E. Kalmbach, R. W. Furness and R. Griffiths. Short- and long-term effects of increased egg production on female body condition

3.1 Abstract

In recent years is has become clear that, contrary to previously established views, egg production can incur substantial costs, particularly for female birds. Egg removal is thus increasingly being used in experimental studies to elevate parental effort and investigate the consequences on present and future reproduction. While most studies focus on life-history parameters, only very few have reported physiological consequences of increased egg production, and fewer still over more than one breeding season. Through egg removal, we induced female great skuas, *Catharacta skua*, to lay six instead of the normal two eggs. In experimental and control females we measured several aspects of body condition between two and eleven days after clutch completion in the experimental and in the following year. As well as using the most common measure of body condition, i.e. weight corrected for body size, we also determined condition of the main protein store, the breast muscle, and investigated haematological values (red blood cell count, haematocrit, and mean corpuscular volume). Nine days after clutch completion females were in significantly poorer body condition after producing six rather than two eggs for all measures of condition we investigated. Even though only haematocrit values improved significantly within two weeks after production of the last egg, overall condition of experimental females improved slightly between two and eleven days after clutch completion. One year after increased egg production, none of the individual parameters differed between control and experimental females. However, when combining all aspects of condition there was a strong tendency of the experimental females to still show reduced values. Despite significant improvement of body condition of experimental females by the following year, there was a strong correlation of individual condition between years. These results present evidence on a physiological level that egg production is expensive for females. It is likely that reduced reproductive performance after increased egg production effort found in other studies is mediated by physiological costs to body condition. The fact that relative condition within a group of females was highly correlated between two years of very different environmental conditions suggests that components of body condition are characteristic for individuals.

3.2 Introduction

Over the last few years, several studies on the costs of egg production in birds have changed the way it is viewed by biologists. While previously, it had been thought that the energetic and fitness costs involved in egg production are minor compared to other demands of reproduction, it is now becoming clear that egg production incurs a substantial cost (Monaghan *et al.* 1998; Nager *et al.* 2001; Visser & Lessells 2001). The main costs of reproduction used to be attributed to the chick rearing period (Roff 1992; Stearns 1992). Many studies therefore employed brood enlargement to experimentally test the idea of reproductive costs (Tinbergen & Daan 1990; Monaghan & Nager 1997). However, since it is becoming more likely that much of this cost is borne during egg production, experimental studies are now increasingly employing egg removal to achieve a higher reproductive effort by parents (Heaney & Monaghan 1995; Monaghan *et al.* 1998; Nager *et al.* 1999; Kalmbach *et al.* 2001). Most studies have looked at the life history implications of this elevated investment in the experimental year (Milonoff & Paananen 1993; Heaney & Monaghan 1995; Monaghan *et al.* 1998), and some also investigate the following season (Nager *et al.* 2001; Visser & Lessells 2001). Only few have tried to measure the physiological consequences of increased egg production for females (Oppliger *et al.* 1996; Monaghan *et al.* 1998). It is most likely that the trade-off between current and future reproduction is mediated through some physiological process. In iteroparous breeders parental condition has an important influence on reproductive decisions (Clutton-Brock 1991; Bolton *et al.* 1992; Weimerskirch *et al.* 1997). It is therefore interesting to follow the recovery of body condition after increased egg production, particularly in long-lived species. However, so far no study has been published which investigates the body condition of females in the year following elevated egg production effort.

To investigate the physiological consequences of elevated investment into egg production, we experimentally increased the number of eggs laid by female great skuas, *Catharacta skua*. Great skuas are long-lived seabirds with high adult survival rates (Ratcliffe *et al.* 2002), which normally lay a clutch of two eggs (Ratcliffe & Furness 1999). Great skuas are known to lay up to two replacement clutches after clutch loss (Furness 1987). Through egg removal we induced the birds to lay three times the normal number of eggs. After production of the last egg we measured several parameters related to female body condition. Even though a deceptively simple concept, and one widely used in ecological studies, body condition still remains difficult to define and measure non-destructively (Brown 1996; Green 2001). In order to get a comprehensive picture, we employed three different methods, which have been used previously to assess body

condition. (i) We measured mass corrected for structural size (body mass index, BMI). This is the most widely used measure by field biologists, and is thought to mainly represent the amount of lipid reserves (Zwarts *et al.* 1996; Hocken 2000). (ii) Following the method described for lesser black-backed gulls *Larus fuscus* by Bolton *et al.* (1991), we took a profile of the flight muscle and calculated a pectoral muscle index. The flight muscle in birds represents the largest single protein store, and has been shown to be an important protein source for females during egg production (Jones & Ward 1976; Houston *et al.* 1983; Houston *et al.* 1995b). (iii) We also measured a range of haematological values for red blood cells: packed cell volume (PCV or haematocrit), total red blood cell count (RBC), and mean corpuscular volume (MCV). The packed cell volume is the most widely reported haematological value and reflects the extent and efficiency of oxygen uptake and transfer to tissues (Ots *et al.* 1998). Extremely low PCV values can represent anaemia, and very high ones show dehydration (Campbell 1988). The PCV is influenced both by the number (red blood cell count, RBC) and size (mean corpuscular volume, MCV) of red blood cells, and more detail about the age and turnover of blood cells can be obtained from these latter measures (Bearhop *et al.* 1999). In a healthy bird, only a small proportion of erythrocytes is immature, reflecting the normal rate of cell replacement. Immature erythrocytes are slightly larger in birds than mature ones, and a relatively higher MCV value therefore reflects a high rate of erythropoiesis.

The body condition measurements of females after increased egg investment are compared to those of control birds at the same time span after normal clutch completion. To record whether body condition was being fully recovered between breeding seasons, we recaptured experimental females in the year following the increased egg production. Because we measured the same individuals in consecutive years, one 'bad' and one 'good' year, we can also investigate the consistency of body condition under varying circumstances. Body condition is thought to reflect the intrinsic quality of a bird (Wendeln & Becker 1999; Hasselquist *et al.* 2001). Body mass for example, has been found to be repeatable for individuals between years (Weimerskirch *et al.* 1997; Wendeln & Becker 1999). If condition is indeed a characteristic connected to an individual's quality, we expect it to be repeatable in its relative rank within a group.

3.3 Methods

The study was carried out at a large colony of great skuas in Foula, Shetland (60°08'N, 2°05'W), in the breeding seasons of 2000 and 2001. The experimental group in 2000 consisted of 55 pairs, from which every new egg was removed within 24 hours of

laying. Some of the experimental birds were already colour-ringed, and most of the un-ringed birds could be identified from individual plumage markings. The egg removal (under licence from SNH) induced the birds to lay further eggs. When a sixth egg was laid, it was left in the nest to be incubated by the parents. On the next day, a dummy egg was added to the nest. This procedure was intended to prevent the females from producing a further egg, to reduce risk of total clutch loss, and to strengthen the ties from the parents to the clutch. However, despite the dummy egg, 13 females produced a seventh egg. In these nests, the dummy egg was removed.

Of the 55 experimental females in 2000, 40 were caught on the nest after production of the last egg. Eight were trapped two days after clutch completion (a.cl.c.), and a further 32 were caught between 7 and 11 days after the last egg had been laid. In 2000, 15 control females, which had produced the normal clutch of two, were also caught between 7 and 11 days a.cl.c. Return rates in 2001 did not differ between experimental and control females (Kalmbach *in prep*). Of the 40 experimental females that had been caught in 2000, 15 were re-trapped in 2001, the year following the egg removal manipulation. They had all laid a normal clutch of two eggs, and were caught 7 to 11 days a.cl.c. An additional 32 control females were caught in 2001.

When caught, each bird was first weighed and a blood sample was taken from the tarsal vein (under licence from the British Home Office) into Monovette® containers which contained EDTA as anticoagulant. Additionally, blood was collected in two heparinised capillaries for haematocrit analysis. Several size measurements were then taken: wing, tarsus, keel and head-bill length. Finally, a profile of the pectoral muscle was taken with solder wire, following the method described by Bolton *et al.* (1991). The previously un-ringed birds were given an individual colour combination. All blood samples were analysed within a maximum of 5 hours after collection. To determine the total red blood cell count (RBC), the cells were stained with Natt and Herrick solution and counted in an improved Neubauer counting chamber. The capillaries were spun for 10 minutes at 10000 rpm, and the proportion of red blood cells was measured to the nearest 0.1 mm using vernier callipers. MCV was calculated as 1000 x (PCV / RBC 10^{12}/l) (see Campbell 1988 for haematological methods). All three haematological parameters are analysed separately, but also combined in a principal component analysis. The first factor of the PCA was extracted and used to represent the overall haematological condition of the bird (referred to as 'blood').

As a measure of body size, the first factor extracted by a principal component analysis on the four measures of size (wing, tarsus, keel, head-bill) was used. Body mass

was regressed over body size ($r_{Pearson}$ = 0.579, $p < 0.001$, n = 53), and the residuals were used as an index of body condition (body mass index, BMI). It has repeatedly been suggested that model II regressions might be more appropriate to describe the functional relationship between mass and size (McArdle 1988; Green 2001). All tests involving the use of residuals were therefore conducted with residuals from both an ordinary least square regression (OLS) and a reduced major axis regression (RMA). None of the results differed between the two methods, and, as expected, the residuals from both methods are highly correlated ($r_{Pearson}$ = 0.886, $p < 0.001$, n = 53). When directly comparing mass / size between groups, an ANCOVA was used (see García-Berthou 2001). Body size was included as the covariate, and year or treatment were integrated as factors. Since an ANCOVA is also based on the OLS method, we decided to only present the results of the OLS regression for the body mass index. Residuals for all females were calculated against the regression line derived from the control birds only. Since we expect the experimental birds to be in poorer condition, including their values in the calculation of the mass - size relationship would bias it away from the expected 'normal' relationship. The effect of increased egg production would therefore be underestimated.

Bolton *et al.*'s (1991) method for estimating absolute lean pectoral muscle weight was derived for lesser black-backed gulls. Without carcass analysis it is not possible to know whether the same relationship would apply for great skuas. We therefore only computed an index of breast muscle volume. To derive this pectoral muscle index (PMI), an area 3 cm under the highest point of the profile trace (equivalent to area (ii) in Bolton *et al.* 1991, fig. 2) was measured. The value for this area was then multiplied by the keel length, in order to correct for size. We used keel length, because it is most closely related to the actual volume of the pectoral muscle. Also, Bolton *et al.* (1991) found a very high correlation between the value of area (ii) X keel length with actual lean dry weight of the muscle ($r = 0.747$, $p \leq 0.000$, Bolton *et al.* 1991, p. 268). We are therefore confident that our PMI presents a meaningful measure in relation to true pectoral muscle condition.

Finally, measures of the three different types of condition (lipid stores, protein stores and haematology) were combined into one overall measure of condition. This is represented by the first factor of a principal component analysis. This PCA included the 'blood' value, the body mass index, and the PMI. The use of the 'blood' value (the scores for the first factor of a haematological PCA) for the calculation of overall condition, was preferred over the inclusion of the three original values, in order to avoid bias towards haematological condition.

All statistical procedures were carried out using SPSS for Windows, version 10.0. When employing multiple t-tests, sequential Bonferroni corrections were carried out to adjust significances (Sokal & Rohlf 1995, p. 241).

3.4 Results

Condition of control females in the two years

No differences in any of the condition parameters were found between control females in 2000 and 2001 (tab. 3.1). For further analysis, all control females were thus pooled into one group, against which experimental birds are compared. Additional to creating a larger sample size, this has the advantage that comparison of experimental females from both years is against the same reference group. Knowing that the control birds' condition was not different between years shows that any differences in the experimental group are unlikely to be due to annual variation.

Table 3.1: Condition parameters of control females in 2000 and 2001. Values are presented as mean ± SD (n). Results of t-tests, and for comparison of the mass/size index results of an ANCOVA with year as factor, are also given.

		2000	2001	t-test / ANCOVA
Blood	PCV	0.45 ±0.02 (15)	0.46 ±0.03 (32)	$t_{45} = 0.96$ $p = 0.342$
	RBC	241.6 ±36.0 (15)	251.0 ±24.7 (32)	$t_{45} = 1.05$ $p = 0.300$
	MCV	1.89 ±0.22 (15)	1.84 ±0.20 (32)	$t_{45} = 0.80$ $p = 0.431$
	Factor 1 'blood'	0.35 ±0.84 (15)	0.29 ±0.57 (31)	$t_{44} = 1.16$ $p = 0.254$
Body mass index		-3.63 ±67.2 (14)	6.08 ±66.3 (32)	ANCOVA year: $F_{1,46} = 0.532$, $P = 0.470$
PMI (x 10^3)		62.8 ±6.4 (15)	63.1 ±5.5 (32)	$t_{45} = 0.14$ $p = 0.891$
'condition'		0.34 ±0.90 (14)	0.60 ±0.84 (31)	$t_{43} = 0.93$ $p = 0.360$

Female condition after producing six eggs

After the production of six (or seven) eggs, females showed significantly lower values for all condition parameters, with the exception of mean corpuscular volume, MCV (fig. 3.1). Experimentals were lighter for their size, had a smaller pectoral muscle and had fewer red blood cells, which is also reflected in an overall poorer score for 'condition' (tab. 3.2). This difference in condition exists between control females and both groups of experimental females, i.e. the ones caught two days and 7-11 days a.cl.c. Within the experimental group, condition did not differ significantly between 2 days and 7-11days a.cl.c. Even though mean values of experimentals 2 days a.cl.c. are all lower than for the ones caught later, this difference only approaches significance for the haematocrit (PCV, tab. 3.2). An ANCOVA of changes in condition over number of days after clutch completion with treatment (control or experimental) as a fixed factor, yields the same result. Treatment is highly significant for all parameters, indicating a poorer condition of experimental birds. Only the PCV is detected as changing significantly over time, i.e. a significant increase of the haematocrit took place during the two weeks immediately after production of the last egg (ANCOVA with PCV as dependent variable, days after clutch completion as covariate ($p = 0.005$), and treatment as factor ($p < 0.001$)).

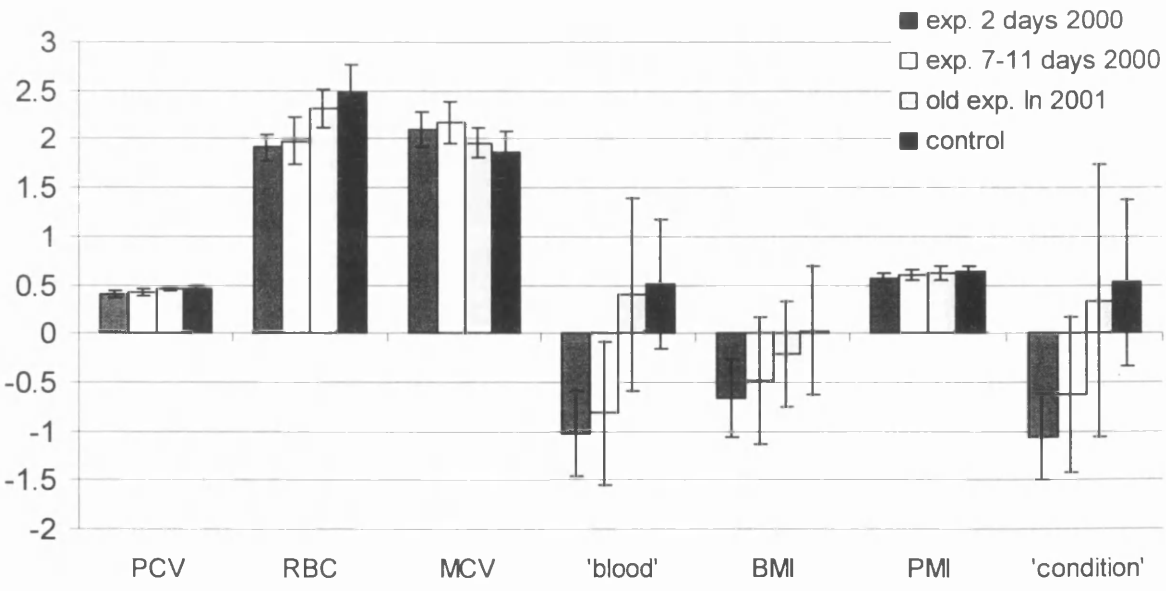

Figure 3.1: Values of different condition parameters for females from different treatment groups: experimental females in 2000 (divided into those caught 2 days a.cl.c. and 7-11 days), experimentals in the year following the manipulation, and control females pooled from both years. Values have been scaled to the same size by the following factors: total red blood cell count (RBC) ÷100; body mass index ÷100; pectoral muscle index $\div 10^6$. Error bars denote ± SD.

Even though not significantly different individually, the means of all measured parameters are lower for 2 day experimentals than for 7-11 day ones (tab. 3.2). When employing a binomial test to the direction of means, this becomes a significant trend (PCV, RBC, body mass index, PMI, and 'condition', $p = 0.031$; assuming an equal probability for the mean being higher or lower if the two groups are truly in the same condition). We excluded MCV from this comparison, as a lower value does not necessarily relate to poorer condition. MCV did not follow the pattern of the other parameters, since control birds have the lowest value, 7-11 day experimentals the highest, with 2 day experimentals lying in between (tab. 3.2). Since MCV reflects the age of red blood cells, this indicates that control birds had on average the oldest ones, while 7-11 day experimentals had a high proportion of immature erythrocytes in their blood.

Table 3.2: Mean values of condition parameters for experimental females 2 and 7-11 days a.cl.c., and control females after 7-11 days. Values are presented as mean ± SD (n). Numbers at the head of columns refer to the results of the Tukey test. Results of ANOVAs and Tukey's post-hoc test are also given. The post-hoc results highlighted in bold represent the comparison between control and 7-11 day experimental females. All significant results remain significant when sequential bonferroni correction is applied.

		1 **Control 7-11 days**	2 **Exp. 2 days**	3 **Exp. 7-11 days**	ANOVA / ANCOVA	Tukey test
Blood	PCV	0.46 ±0.03 (47)	0.40 ±0.03 (8)	0.42 ±0.03 (32)	$F_{2,86} = 22.287$ $P = 0.000$	1-2: 0.000** **1-3: 0.000**** 2-3: 0.095
	RBC	248.0 ±28.7 (47)	190.9 ±13.9 (8)	198.0 ±25.0 (28)	$F_{2,82} = 38.809$ $p = 0.000$	1-2: 0.000** **1-3: 0.000**** 2-3: 0.784
	MCV	1.89 ±0.22 (47)	2.10 ±0.18 (8)	2.17 ±0.21 (28)	$F_{2,82} = 21.160$ $P = 0.000$	1-2: 0.007* **1-3: 0.000**** 2-3: 0.715
	Factor 1 'blood'	0.51 ±0.67 (46)	-1.02 ±0.43 (8)	-0.81 ±0.73 (28)	$F_{2,81} = 41.779$ $p = 0.000$	1-2: 0.000** **1-3: 0.000**** 2-3: 0.714
Body mass index		3.13 ±66.0 (46)	-65.93 ±39.9 (8)	-48.00 ±63.9 (32)	ANCOVA treatm $F_{2,86}$ = 7.882; p= 0.001	
PMI (x 10^3)		63.0 ±5.7 (47)	56.8 ±5.2 (8)	59.9 ±4.7 (32)	$F_{2,86} = 6.313$ $p = 0.003$	1-2: 0.009* **1-3: 0.036*** 2-3: 0.305
'Condition'		0.52 ±0.86 (45)	-1.06 ±0.43 (8)	-0.59 ±0.79 (28)	$F_{2,80} = 23.792$ $p = 0.000$	1-2: 0.000** **1-3: 0.000**** 2-3: 0.323

Recovery of body condition between years

In the year following increased egg production only the total red blood cell count of old experimentals was still significantly lower than the value of control birds (tab. 3.3), even though after correcting for multiple testing this difference is not significant anymore. Even though the mean values of all other condition measures (except MCV) were also lower for old experimentals than for control birds (fig. 3.1), none of these differences were significant (tab. 3.3). At a first glance it appears that body condition was recovered between breeding seasons to the level of unmanipulated birds. However, the fact that all mean values were lower for old experimentals implies that there might still have been a subtle difference in condition. When employing a binomial test to the direction of means, this becomes a significant trend (PCV, RBC, body mass index, PMI, and 'condition', $p = 0.031$; assuming an equal probability for the mean being higher or lower if the two groups are truly in the same condition). These results indicate that old experimental females still had a slightly poorer overall condition one year after increased egg production.

Table 3.3: Condition parameters of old experimental females in 2001 and control females. Results of t-tests are given and for comparison of mass/size results of an ANCOVA with treatment as factor. After applying Bonferroni correction, no differences were significant anymore.

		Old experimentals	**Controls**	t-test / ANCOVA
Blood	PCV	0.45 ±0.02 (15)	0.46 ±0.03 (47)	$t_{60} = 0.30$ $p = 0.765$
	RBC	232.3 ±18.8 (15)	248.0 ±28.7 (47)	$t_{60} = 2.451$ $p = 0.019$
	MCV	1.96 ±0.16 (15)	1.89 ±0.22 (47)	$t_{60} = 1.781$ $p = 0.080$
	Factor 1 'blood'	0.40 ±1.0 (15)	0.51 ±0.67 (46)	$t_{59} = 0.489$ $p = 0.627$
Body mass index		-21.70 ±54.4 (15)	3.13 ±66.0 (46)	ANCOVA treatm: $F_{1,61} = 1.672$, $p = 0.201$
PMI (x 10^3)		62.1 ±6.5 (15)	63.0 ±5.7 (47)	$t_{60} = 0.497$ $p = 0.621$
'condition'		0.33 ±1.40 (15)	0.52 ±0.86 (45)	$t_{58} = 0.496$ $p = 0.626$

Individual birds improved body condition significantly from the year with extra egg production to the following season. Total red blood cell count, haematocrit, and the overall condition increased significantly from 2000 to 2001 (tab. 3.4). MCV decreased, which signifies a reduction in the proportion of immature red blood cells. Both the PMI and the body mass index had an increasing tendency between the two years, but the increase is marginally below the significance value of 5 %. Even though condition changed significantly, it was highly correlated between the two years (fig. 3.2). Body mass index and total red blood cell count showed the strongest inter-annual correlation, but 'blood', PMI and overall condition were also significantly correlated (fig. 3.2). Haematocrit values improved significantly and tended to be consistent between years, and only MCV does not show an inter-annual relation (fig. 3.2).

Table 3.4: Condition parameters of experimental females in the experimental (2000) and in the following year (2001). Results of paired t-tests are given. All significant results remain significant after sequential Bonferroni correction.

		2000	2001	Paired t-test
Blood	PCV	0.41 ±0.03 (15)	0.45 ±0.02 (15)	$t_{14} = 4.799$ $p = 0.000^{**}$
	RBC	194.5 ±24.4 (14)	232.5 ±19.5 (14)	$t_{13} = 8.872$ $p = 0.000^{**}$
	MCV	2.15 ±0.25 (14)	1.95 ±0.16 (14)	$t_{13} = 2.888$ $p = 0.013^{*}$
	Factor 1 'blood'	-0.92 ±0.7 (14)	0.41 ±1.0 (14)	$t_{13} = 7.269$ $p = 0.000^{**}$
Body mass index		-43.70 ±65.2 (15)	-20.70 ±54.4 (15)	$t_{14} = 1.663$ $p = 0.118$
PMI (x 10^3)		58.9 ±5.5 (15)	62.1 ±6.5 (15)	$t_{14} = 2.126$ $p = 0.052$
'condition'		-0.64 ±0.76 (14)	0.34 ±1.45 (14)	$t_{12} = 3.037$ $p = 0.010^{*}$

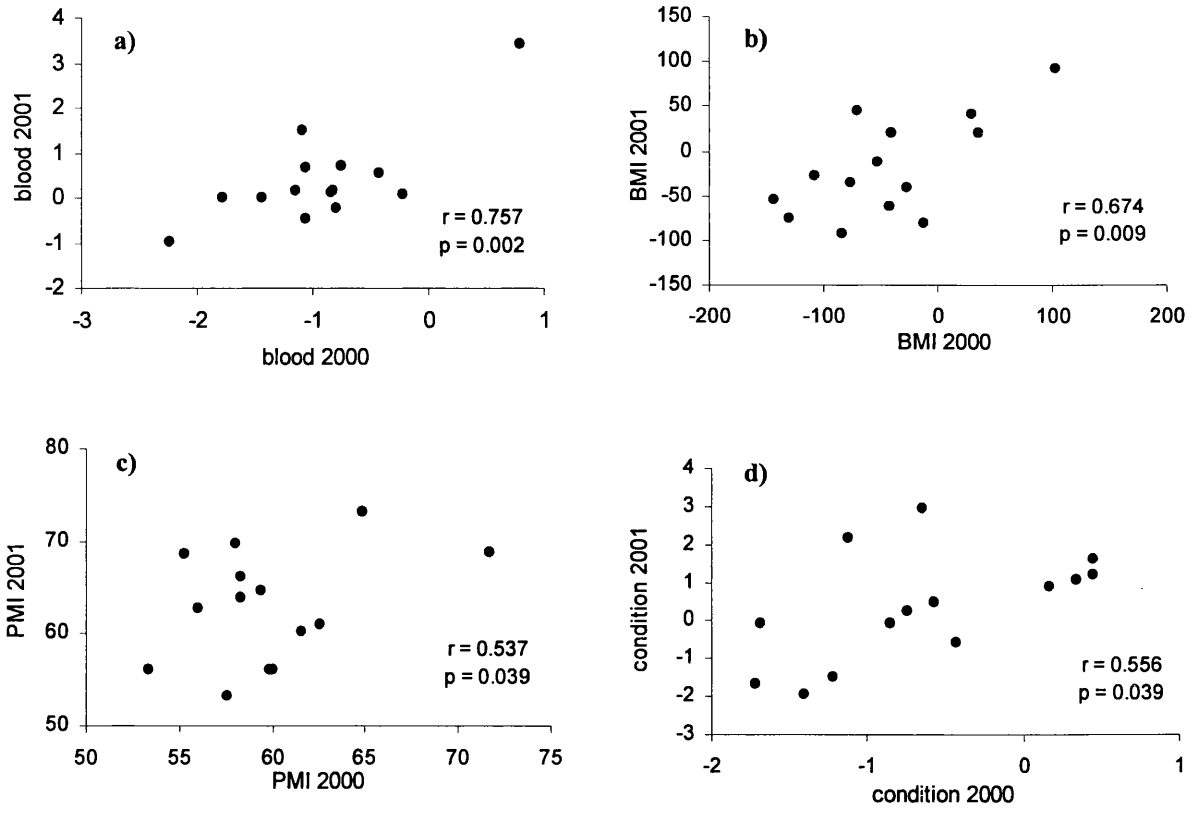

Figure 3.2 a-d: Correlation of condition parameters for individual experimental females in 2000 and 2001. a) PCA factor 1 score for 'blood', b) body mass index, c) pectoral muscle index, and d) PCA factor 1 score for overall 'condition'. Values of correlation coefficients and significance levels are given in the figures. Coefficients for individual haematological parameters are: haematocrit: $r = 0.49$, $p = 0.063$; total red blood cell count: $r = 0.756$, $p = 0.002$; mean corpuscular volume: $r = 0.331$, $p = 0.248$.

3.5 Discussion

Our results clearly demonstrate that extra egg production is indeed demanding for female birds, and affects many different aspects of their body condition. Female great skuas, which had produced six eggs, rather than the normal clutch of two, had significantly lower haematological values, were lighter for their size, and had a lower pectoral muscle index. Despite the fact that we measured condition over a week after production of the last egg, experimental females were still in a distinctly poorer condition than control birds. This shows that the females do not easily recover from the loss of condition after increased egg production. Even though a positive trend of recovery from two days to nine days after clutch completion could be detected, the improvement was not substantial. This suggests

that poor condition at the end of egg production will be carried on throughout the remaining breeding season.

The lower weight and pectoral muscle volume reflect an increased depletion of both lipid and protein reserves over the period of extended egg laying (Jones & Ward 1976; Golet & Irons 1999; Durell *et al.* 2001;). Pectoral muscle tissue has previously been shown to be an important protein source for egg production (Houston *et al.* 1995a; Monaghan *et al.* 1995). The decrease in muscle tissue is therefore not surprising. Previous to and during incubation male great skuas undertake most of the foraging for a pair (Catry *et al.* 1999a). This might allow females to reduce their pectoral muscle volume without severe consequences on her nutritional intake following a decrease in flight ability. The depletion of fat reserves is probably only to a lesser degree due to a direct incorporation into developing eggs. Lipid reserves are not connected to a female's egg production capacity to the same extend as protein (Houston *et al.* 1983; Bolton *et al.* 1993). A large part of the energy stored as lipids might have been used for the maintenance of the female's metabolism, particularly during reduced foraging ability in addition to the energetically demanding process of egg formation (Carey 1996).

During egg laying, a reduction of red blood cells has been observed in females of several species, and different reasons for this have been suggested (Bell *et al.* 1965; Jones 1983; Morton 1994; Merino & Barbosa 1997). Morton (1994) proposed that the apparent decline in RBC is only a side effect of the increased influx of protein, lipid and calcium into the plasma. This would result in a fixed decline of RBC for the time of increased protein metabolism. However, the fact that RBC and PCV were much lower after producing six rather than two eggs suggests that the reduction is proportional to egg laying effort, rather than a fixed decline. More evidence comes from the MCV values, which show a high proportion of immature red blood cells in experimental birds. Coupled with the much lower total number of red blood cells present, this suggests the onset of a high haemopoietic activity after production of the last egg. The values from experimental females just two days after clutch completion support this: the total red blood cell count is at its lowest, and a lower proportion of immature erythrocytes is present than a week later. This suggests that the increased haemopoietic activity had just started and the full effects of it, i.e. a high proportion of immature red blood cells, is not detectable yet. It seems clear that the decline of red blood cells is due to a continuous breakdown of old cells without replacement. This reduced erythropoietic activity during egg laying might be due to increased oestrogen levels during follicle maturation (Jones 1983; Prinzinger & Misovic 1994). Oestrogen is being produced by maturing follicles and is thought to be

erythropoiesis inhibiting. Alternatively, red blood cell formation might be suppressed and nutrients are diverted to growing yolk follicles (Jones 1983). In both scenarios, a prolonged period of egg laying would exacerbate the decline in red blood cells. Considering the depletion of body reserves we report, it is most likely that a combination of these two factors is responsible for the observed lack of red blood cell formation during egg laying. There has been much debate over the worth of haematocrit values as a general measure of condition (Dawson & Bortolotti 1997; Bearhop *et al.* 1999). However, it seems clear that values outside the normal range are detrimental, since they are connected to anaemia, illness or dehydration (Campbell 1988; Dawson & Bortolotti 1997). The lower haematocrit values of experimental females certainly signify a compromised efficiency in oxygen uptake and transfer to the tissues. In some birds haematocrit was so low, that it actually represents anaemia (Campbell (1988, p. 7) gives a value of 0.35 or lower for anaemia; our lowest values was 0.35). Campbell's (1988) value is based on caged birds and it is likely that in the wild oxidative demands on birds are much higher, and the actual value representing anaemia is an overestimation. In recent years, it has been suggested that the immune system might provide an important physiological link between life history trade-offs (Oppliger *et al.* 1996; Sheldon & Verhulst 1996). It would have been interesting to include aspects of immunocompetence in our condition measurements. Unfortunately, for most immunological tests, individuals need to be re-captured after several days, which is not practicable with individual great skuas. However, an overall reduction in condition is likely to have negative effects on the immune system as well (Keymer & Read 1991; Lochmiller *et al.* 1993; Hasselquist *et al.* 2001).

Haematological values are generally regarded as reflecting recent events and changes in a bird's situation, such as workload or condition (Ots *et al.* 1998; Potti *et al.* 1999). It is therefore surprising to find that the number of red blood cells was the one parameter for which we could still detect a difference between experimental and control females after one year. Even though individually, all other condition parameters did not show significant differences, in combination they suggest a slightly poorer condition of females one year after increased egg production. Since adult body condition can have an important influence on reproductive decisions (Clutton-Brock 1991; Bolton *et al.* 1992; Weimerskirch *et al.* 1997), this is a strong indication for a trade-off between reproductive investment and maintenance of adult body condition, and thereby future reproductive success (Nager *et al.* 2000; Nager *et al.* 2001). Our results demonstrate on a physiological level, what other studies have shown in a life history context: increased egg production does not only have immediate negative implications, but also possible carry-over effects to

the following year (Nager *et al.* 2001; Visser & Lessells 2001). In long-lived and iteroparous birds, preservation of adult long-term condition is expected to be of higher priority than a single breeding event (Weimerskirch 1992; Mauck & Grubb Jr 1995). We would therefore expect females to stop laying once their own long-term recovery is being jeopardised. Our results indicate that this might not have been the case.

Condition is thought to reflect individual quality (Wendeln & Becker 1999; Hasselquist *et al.* 2001). Since quality is often assumed to be an intrinsic (possibly genetically determined) characteristic of individuals, condition should equally be so. Our data give strong evidence that a component of body condition could indeed be an individual characteristic. Condition in experimental females was highly correlated between the two study years. This is true not only for measures which are connected to body size (BMI and PMI), but also for the size-unrelated haematological parameters. Considering that, due to our experimental manipulations, circumstances were very different in the two years, the inter-year correlation is remarkable. Birds which were in poorer condition during the experiment, were also in poorer condition under non-experimental circumstances. Surprisingly, even RBC, which is thought to be a rapidly changing parameter, appears to be characteristic for individuals between years. However, despite the consistency of relative condition over two very different seasons, over a longer period of time it might vary considerably. Laying dates, as an aspect of quality (Catry *et al.* 1998), have been found to be repeatable for individual great skuas over a period of two to three years, but were not repeatable over an extended period of four to five years (Catry *et al.* 1999b). Environmental conditions can affect the state of individuals to a different extent (McNamara & Houston 1996). This could highlight inter-individual differences within the same group over an increasing number of years and lead to decreasing repeatabilities. The fact that all physiological measurements we investigated showed the same trends within and between years (i.e. poor after extended egg production and recovery to following year), strongly indicate that they all measure an aspect of the same property, namely body condition.

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Chapter 4

Does supplementary food enhance egg laying capacity and egg quality?

Manuscript:

E. Kalmbach, P. Catry, K. Ensor, R. Griffiths, S. Waldron and R. W. Furness. Does supplementary food enhance egg laying capacity and egg quality? An experimental study in the great skua, *Catharacta skua*.

4.1 Abstract

As egg production has been shown to be nutritionally demanding for female birds, experimental studies increasingly employ the provisioning of supplementary food in order to enhance egg production ability. Enhanced egg laying capacity can result in advanced laying dates, increased size of eggs or number of eggs produced. Egg quality in terms of nutrient composition might also be affected following the provisioning of supplementary food. Few studies have investigated the consequences of supplementary food on egg composition and have compared the eggs to those from non-supplemented control birds. We conducted an egg removal experiment coupled with supplementary feeding in the great skua *Catharacta skua,* and analysed the effects of enhanced food availability on the composition of eggs from extended laying sequences. Additionally we employed stable isotope analysis to trace the incorporation of supplementary food into eggs. We used food from a terrestrial source which had different ^{13}C and ^{15}N signatures from the mainly marine food source of the skuas. Timing of laying, egg sizes, and number of eggs were not influenced by feeding treatment. Egg size and quantity of nutrients declined over the laying sequence, the only difference between the treatment groups being that the decline of lipid content over the sequence was steeper in clutches from un-fed females. In both groups the proportion of lipids increased slightly with increasing laying order. Provisioning of supplementary food had a stronger effect on clutches from late laying females than from early females. The rate of nutrient decline was slower in late clutches of fed birds than in late clutches of un-fed ones. $\delta^{15}N$ and $\delta^{13}C$ values of eggs from fed birds were more depleted than eggs from controls, indicative of the incorporation of supplementary food into both the protein and lipid fractions of the eggs. Even though none of the egg components had a significant effect on embryo sex, there was a tendency of the total lipids to be higher with male embryos ($p = 0.065$). Thus, supplementary food changed the way females provisioned their eggs, but only slightly. Lipid content appeared to be the aspect that was most affected. Good environmental conditions during our study year might have masked stronger effects of the supplementary food on egg production when food is more limited.

4.2 Introduction

The provisioning of supplementary food has become a widely used technique for experimental life history studies in birds (Blount *et al.* 2001; Clifford & Anderson 2001; Kalmbach *et al.* 2001; Verboven *et al.* 2001). The majority of these studies are conducted throughout the breeding season. The aim of the supplementary food is generally to reduce parental effort during the reproductive period or to enhance parental breeding capacity (Wernham & Bryant 1998; Wiehn & Korpimaki 1998; Moreno *et al.* 1999; Kalmbach *et al.* 2001). Most studies have focussed on the chick rearing stage, which has traditionally been viewed as the most demanding phase of reproduction (Monaghan & Nager 1997). However, studies focussing only on the nestling stage have produced mixed results, and the scope of research into reproductive constraints has widened in recent years and now often includes the period of egg synthesis, laying and incubation (Monaghan *et al.* 1998; Nager *et al.* 2001; Visser & Lessells 2001). During the egg laying stage, supplementary food is commonly perceived to enhance egg laying capacity (Bolton *et al.* 1993; Nager *et al.* 1997; Clifford & Anderson 2001). However, the way in which this enhancement manifests itself is not always clear. Some birds change the timing of laying (Arnold 1994; Svensson & Nilsson 1995; Kelly & Van Horne 1997), some change the size of eggs (Foeger & Pegoraro 1996; Ramsay & Houston 1997), or the number of eggs they produce (Arcese & Smith 1988; Bolton *et al.* 1992; Arnold 1994; Nager *et al.* 1997; Clifford & Anderson 2001). In recent years several studies have investigated which nutritional aspect is limiting for egg production (Bolton *et al.* 1992; Selman & Houston 1996; Williams 1996; Nager *et al.* 1997). While it had previously been assumed that the constraint on egg production is mainly energetic (Nager *et al.* 1997), several studies provide evidence that proteins might be limiting (Bolton *et al.* 1992; Houston *et al.* 1995; Williams 1996). Some studies also suggest that the balance of micro nutrients in the diet, such as vitamins, carotenoids or specific amino acids, could be a limiting factor (Selman & Houston 1996; Houston 1997; Blount *et al.* 2001).

Life history studies usually focus on the fate of chicks and parents from supplemented or non-supplemented groups (Svensson & Nilsson 1995; Wernham & Bryant 1998; Nager *et al.* 1999; Verboven *et al.* 2001). Even when supplementary food enhances clutch size, the final reproductive success of the supplemented group is rarely higher than in the control group (Nager *et al.* 1997). This indicates that even though supplemented birds can produce more or bigger eggs, the quality of the eggs might not stay the same. Nutrient composition of eggs can be vital to the success and development of chicks (Hill 1993; Bernardo 1996; Finkler *et al.* 1998). However, only very few studies

have investigated how supplementary food translates into changes in egg contents (Arnold 1992; Hario 1997; Blount *et al.* 2001).

In the present study, we investigated egg composition of supplemented and non-supplemented great skuas, *Catharacta skua.* Great skuas are predatory seabirds, and their diet includes fish as well as other birds and occasional scavenging on other dead animals (Furness & Hislop 1981; Phillips *et al.* 1997; Ratcliffe *et al.* 1998; Bearhop *et al.* 2001). We chose cat food as supplementary food, which consists mainly of animal remains, but also includes small quantities of plant material. It should therefore provide a balanced addition to the natural food source. Nager *et al.* (1997) showed that differences in egg production capacity between supplemented and non-supplemented groups are highlighted by poor circumstances. In this study we created harsh circumstances for the experimental birds by egg removal, which induced females to lay more than the normal clutch size of two. If supplementary food enhances egg laying capacity primarily under stressful circumstances, we expect later eggs in the sequences of the two experimental groups to be more affected than first eggs. We analyse egg composition over the extended laying sequences. Instead of using the additional food for egg synthesis, birds might also take it as replacement of part of their natural food source and therefore forage less, or might use the food to maintain their own body functions. In order to test whether the additional food we provided was incorporated directly into the eggs, we performed a stable isotope analysis on a sub-sample of eggs from the fed and un-fed group. Marine and terrestrial organisms show a distinct stable isotope ratio of carbon (^{13}C / ^{12}C), while nitrogen stable isotope ratio (^{15}N / ^{14}N) varies more according to trophic status (Hobson 1999). Hobson (1995) showed that the dietary isotopic composition is reflected in isotopic composition of the eggs, with different but consistent fractionation patterns for stable carbon and nitrogen isotopes. The isotopic signature of cat food is distinct from that of the natural food source of great skuas, since it contains almost exclusively terrestrial rather than marine animal products.

4.3 Methods

The study was carried out during May and June 1999 in Foula, Shetland (60°08'N,2°05'W). We used 70 pairs of great skuas for our experiment, which were randomly assigned to the fed or un-fed group. 92 of our study birds were already ringed with unique colour combinations, and the majority of the other 48 could be identified through individual plumage markings. The 38 pairs in the fed group received daily portions of supplementary food from 1 May onwards. This ensured that all fed pairs received food from at least 10 days prior to laying of the first egg (min 10 d, max 25 d before first egg).

We fed them with 400g of tinned cat food (Safeways Savers brand) per pair per day, which contained an average of 1100 kJ, equivalent to approximately 30% of the daily energy need of one pair (Ratcliffe 1993). Since the males undertake most of the foraging and then regurgitate food for the female (Caldow & Furness 2000), it was not critical which of the two partners was present at the time of feeding. In order to avoid risk of theft of food by non-experimental birds, feeding took place in the late evening, a time with generally high territorial attendance and minimal risk of territorial intrusions. All pairs readily accepted the food and, due to the individual markings, we were sure that only target birds took the food.

We conducted daily nest checks prior to and during egg laying and every new egg was removed within 48 hours of laying (usually within 24 hours). The egg removal induced the birds to lay further eggs and was continued until birds stopped relaying. Both partners of the study pairs remained on their territories throughout the egg removal and directly after the experiment. Egg length and breadth was measured and an egg volume index was computed by (breadth^2 x length) / 1000. The fed and un-fed group did not differ in laying date, total number of eggs laid, the average inter-egg interval or the volume of first eggs (Kalmbach *et al.* 2001).

For purposes of sex determination (see Kalmbach *et al.* 2001), eggs were artificially incubated for five days, after which embryos were removed. The remaining egg contents were frozen and stored until further analysis. After thawing, the soft egg contents were separated from the shell and both parts were weighed. Since freezing and thawing of fresh material can alter water content, fresh weights presented here are not 'true' fresh weights, i.e. directly at laying. However, all eggs in our study were treated equally, which allows the comparison of fresh weights within our sample. Contents and shell were then dried at 60 °C until constant weight was achieved. Water content was deducted as the amount of weight lost during the drying process. The dried contents were homogenised and from one half of each sample lipids were extracted in a Soxhlet apparatus, using chloroform as solvent. The remaining part of the sample represented the protein fraction of the egg, and the weight difference between whole and lipid free dry weight gives a measure of the amount of lipid per egg.

For a sub sample of eggs, the lipid containing half was frozen to liquid nitrogen temperature and powdered in an analytical mill. For four females, two each from the fed and un-fed group, six or seven eggs were analysed for $^{13}C/^{12}C$ ($\delta^{13}C$) and $^{15}N/^{14}N$ ($\delta^{15}N$) isotope ratios. Samples were filled into tin cups (4 x 6 mm) and analysed by continuous flow isotope ratio mass spectrometry in a Europa Anca 20-20 C/N/S mass spectrometer.

Each run contained an international standard (Pee Dee belemnite (PDB) for ^{13}C and atmospheric nitrogen for ^{15}N), a laboratory reference sample of known isotopic composition, followed by duplicate egg samples. Isotope ratios are given as permille (‰), calculated from the following formula:

$$\delta^{13}C \text{ or } {}^{15}N = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000$$

with R being the respective ratio of $^{13}C/^{12}C$ and $^{15}N/^{14}N$.

For statistical analysis of volume and composition changes of the eggs occurring over laying sequence, Spearman's correlation coefficient of every parameter over laying sequence was computed for each female. Therefore, irrespective of the total number of eggs laid, each female only contributes one data point to the analysis. All correlation coefficients given are Spearman's rank coefficients, unless otherwise stated. For the comparison of clutches of different sizes, relative position (hereafter simply referred to as position) in the laying sequence was calculated for each egg as $(i - 1) / (i_{max} - 1)$, with i = egg number and i_{max} = number of the last egg. This results in every first egg having the relative position 0, and every last egg occupying position 1, with intermediate eggs occupying positions at equal distances between 0 and 1. To examine the relationship between egg composition and embryo sex, general linear mixed models (GLMM) with a binomial error structure were fitted to the data (Kruuk *et al.* 1999; Schall 1991), using the Genstat 5 package, version 4.1. Embryo sex was included as the dependent variable and the different nutrient components as explanatory variables. To account for the different number of eggs laid by each female, pair identity was included as a random factor. Additionally, a dispersion factor was calculated by the model, to account for extra-binomial variation (see also Kalmbach *et al.* 2001). All other statistical analysis was carried out with the SPSS for Windows, version 10 software package.

4.4 Results

Changes of egg mass and composition over extended laying sequence

First eggs laid by females of the two treatment groups were almost identical (tab. 4.1). The eggs did not differ in either egg mass and volume or in the absolute and relative contents of water, lipid and protein (tab. 4.1). After the first egg, egg size and mass subsequently declined in both treatment groups over the extended laying sequence, as did the quantity of nutrients and water (tab. 4.2). With regard to absolute amounts, the rate of

decline only differed between treatment groups for the lipid fraction, which was steeper in the un-fed group (fig. 4.1). Despite declining egg size and quantities of nutrients, the relative composition of eggs appeared to stay considerably constant throughout the laying sequence in both groups. However, there was an indication that while the relative amounts of water and protein did not change throughout the laying sequence, the relative portion of lipids tended to increase slightly in sequences of fed and un-fed females (tab. 4.2). Thus, eggs towards the end of the laying sequence appear to have had a proportionately higher content of lipid than earlier eggs.

Table 4.1: Egg components of first eggs laid by fed and un-fed females and results of t-tests between the two groups.

	Un-fed (n = 30)	**Fed (n = 38)**	**t-test t_{66} / p**
Volume index	172.4 ± 3.2	175.5 ± 2.3	-0.790 / 0.430
Mass excl. shell (g)	80.95 ± 0.83	82.63 ± 6.74	-0.924 / 0.359
Shell mass (g)	6.07 ± 0.61	6.11 ± 0.54	-0.252 / 0.802
Water (g)	62.24 ± 6.76	63.73 ± 5.62	-0.992 / 0.325
Protein (g)	11.78 ± 1.22	11.98 ± 1.12	-0.689 / 0.493
Lipids (g)	6.93 ± 0.76	6.93 ± 0.66	0.007 / 0.994
% water	76.85 ± 0.96	77.09 ± 1.07	-0.975 / 0.333
% protein	14.57 ± 0.74	14.50 ± 0.71	0.423 / 0.674
% lipids	8.58 ± 0.83	8.4 ± 0.86	0.818 / 0.416

Table 4.2: Rank correlation coefficients for changes in egg composition over laying sequence grouped for fed and un-fed birds. In the one-sample t-test, correlation coefficients were tested against a value of 0, equal to no change over laying sequence. Stars denote significant results after sequential Bonferroni correction. Of the egg contents, only the decline in absolute lipid content differed between treatment groups (see fig. 4.1).

	Coefficient (average, n = 62)	**One-sample t-test t_{61} / p**	
Volume index	-0.6972	-15.404 / 0.000	*
Mass excl. shell (g)	-0.6779	-13.547 / 0.000	*
Shell mass (g)	-0.6100	-10.165 / 0.000	*
Water (g)	-0.6448	-11.799 / 0.000	*
Protein (g)	-0.6782	-14.407 / 0.000	*
Lipids (g)	-0.5495	-10.681 / 0.000	*
% water	-0.0970	-1.487 / 0.142	
% protein	0.0857	1.140 / 0.259	
% lipids	0.1297	2.164 / 0.034	

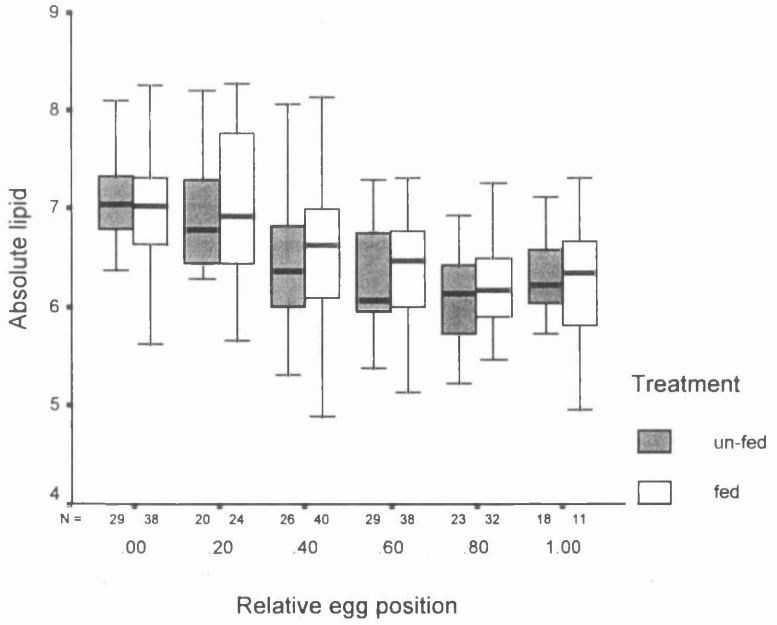

Figure 4.1: Changes of absolute lipid content over relative laying position for fed and un-fed birds. For graphic purposes, relative positions have been grouped into classes between first egg = 0 and last egg = 1. $r_{un\text{-}fed} = -0.7044$, $r_{fed} = -0.4133$, $t_{60} = 3.114$, $p = 0.003$.

Changes in egg composition in relation to lay date and total number of eggs laid

The earlier in the season a female started laying, the more eggs she produced in total (correlation of lay date and total number of eggs: $r = -0.532$, $p < 0.001$, $n = 69$). The rate of decline of several egg components was negatively correlated with lay date in both treatment groups. This indicates that the reduction of egg contents was steeper in sequences which were initiated later in the season. Looking at the decrease of total amounts, this pattern was found for fresh weight of contents (fig. 4.2a), fat content (fig. 4.2b), water content ($r = -0.295$, $p = 0.020$, $n = 62$), and shell weight ($r = -0.314$, $p = 0.013$, $n = 62$), while protein content did not decrease more rapidly in late clutches ($r = -0.098$, $p = 0.450$, $n = 62$). There was also a tendency for a steeper decline in egg volume with a later lay date ($r = -0.241$, $p = 0.059$, $n = 62$). Thus, compared to early clutches, eggs from clutches which were initiated late in the season became smaller and lighter more quickly, which was reflected in the rapid decline of total fat and water content.

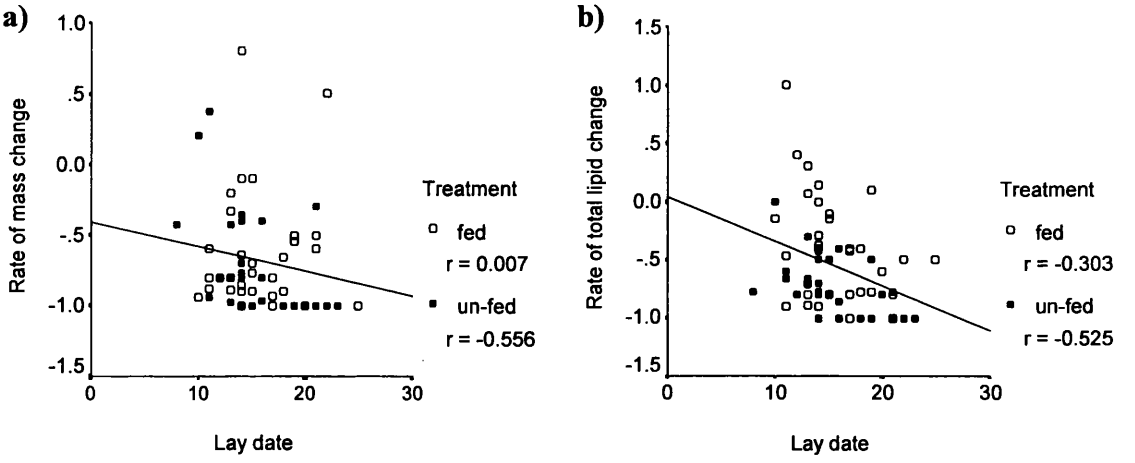

Figure 4.2 a,b: Relationship between the rate of decline of a) mass and b) absolute amount of lipid (represented by the correlation coefficient of change over laying sequence on y-axis) and lay date. a) mass of soft contents ($r = -0.285$, $p = 0.025$, $n = 62$); b) absolute lipid ($r = -0.353$, $p = 0.005$, $n = 62$).

When considering the change of relative egg composition in clutches of early and late laying females, we find some patterns that are different from the change of total quantities. The relative amount of protein declined in sequences laid by early females, and increased in extended clutches of late birds (fig. 4.3a). However, there were some differences in egg composition for early and later females between the fed and un-fed group. While on average, the rate of decline of the lipid fraction did not change with lay date, the pattern over laying date differed between fed and un-fed birds: late un-fed birds

tended to increase the relative amount of lipids over the laying sequence, while fed ones tended to decrease it (fig. 4.3b; GLM with dependent 'lipid change over laying sequence': explanatory variables treatment ($p = 0.043$), lay date ($p = 0.797$) and treatment*lay date ($p = 0.044$)). Similarly, the change of the water proportion throughout a sequence of eggs became steeper in late clutches of un-fed females only (fig. 4.3c). In summary, the decline of egg size and total nutrient quantities over the laying sequence was more pronounced in later clutches of both treatment groups. However, the change of relative egg composition in relation to laying date differed between fed and un-fed birds. The effect of lay date on the rate of egg component changes was less pronounced in clutches of fed birds than in those of un-fed pairs.

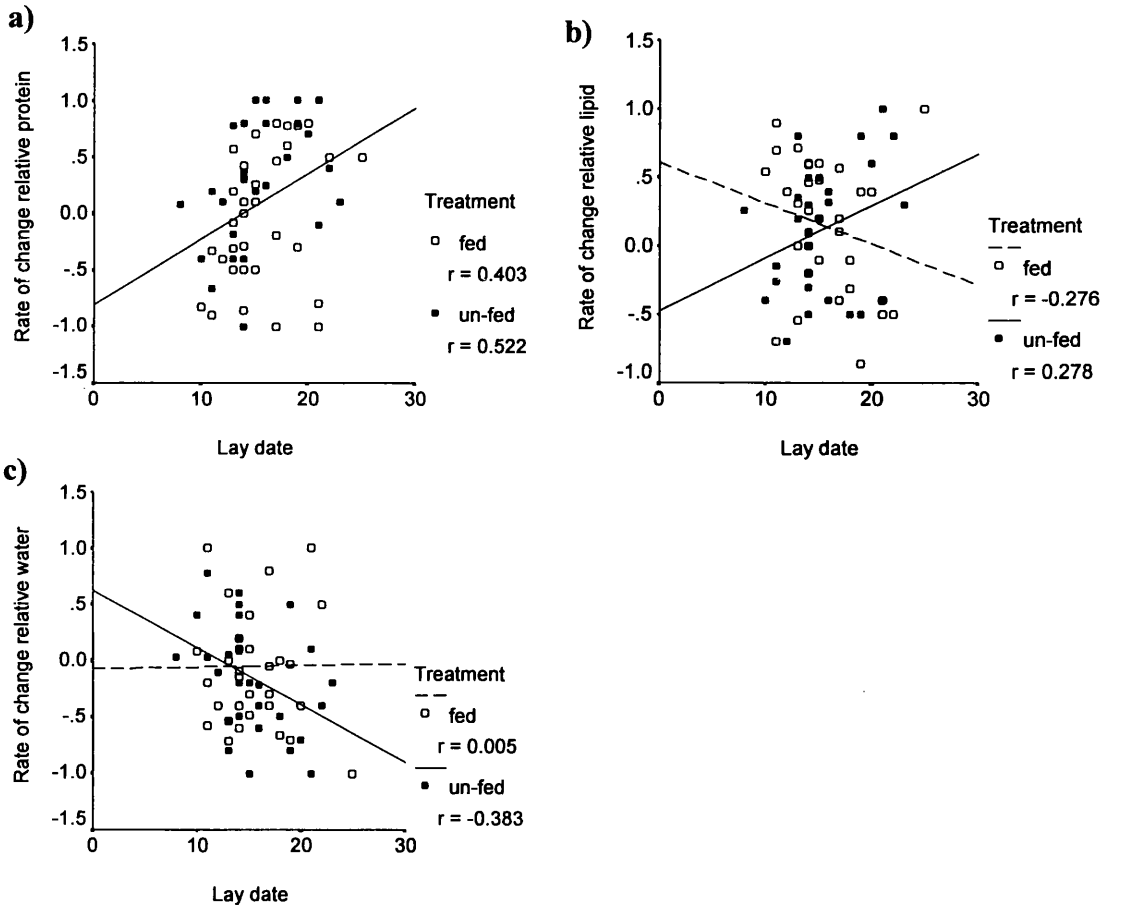

Figure 4.3 a-c: Relationship between the rate of decline of relative amounts of egg components (represented by the correlation coefficient of change over laying sequence on y-axis) and lay date. a) relative protein ($r = 0.433$, $p = 0.000$, $n = 62$); b) relative lipid ($r = 0.003$, $p = 0.981$, $n = 62$; the change over lay date is significantly different for fed and un-fed birds; see text); and c) relative water content ($r = -0.182$, $p = 0.158$, $n = 62$).

Females which produced a large number of eggs, had the capacity to provision their eggs with more fat than those that only produced small clutches. In eggs of large clutches from both treatment groups the relative amount of lipids increased over the laying sequence, and the decline of total lipids was less steep (fig. 4.4). The relative amount of lipids was not only correlated with the total number of eggs a female laid, but also with the size of the first egg she produced. Females which produced large first eggs, were able to increase the relative amount of lipid in the eggs over the laying sequence ($r = 0.282$, $p = 0.029$, $n = 60$). The rate of change of most other components also correlated positively with the total number of eggs laid, however, not significantly so.

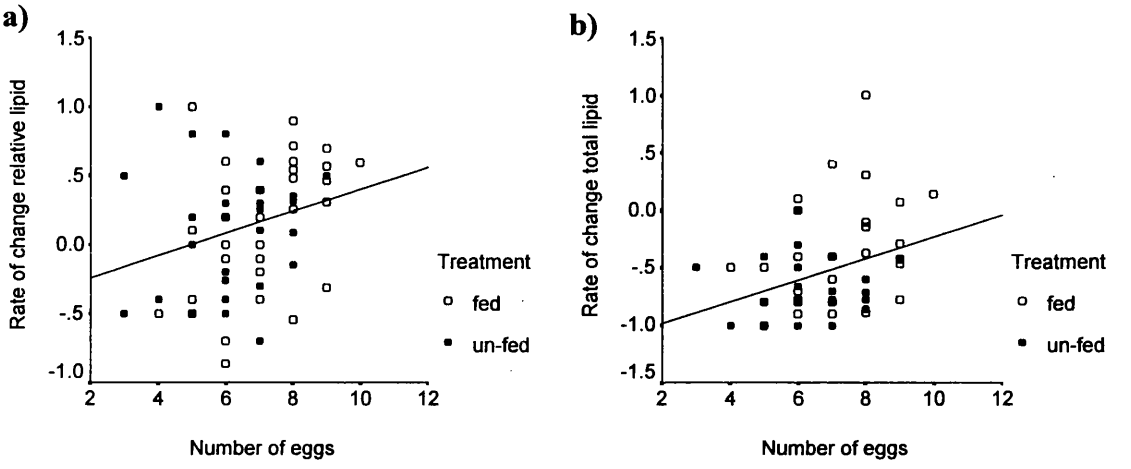

Figure 4.4 a,b: Correlation between the rate of decline in a) relative lipid content ($r = 0.282$, $p = 0.026$, $n = 62$), and b) absolute lipid content ($r = 0.379$, $p = 0.002$, $n = 62$) with the total number of eggs laid for fed and un-fed birds.

Incorporation of supplementary food into eggs – isotopic analysis

Eggs from fed birds showed a different isotopic signature than eggs from the un-fed group, for both carbon and nitrogen stable isotopes (fig. 4.5). Un-fed eggs had a higher enrichment level of ^{15}N (nested ANOVA, between subject effects: treatment $F_{1,24} = 21.601$, $p \leq 0.001$; pair (nested within treatment) $F_{2,24} = 2.806$, $p = 0.084$), and also of ^{13}C (nested ANOVA, between subject effects: treatment $F_{1,23} = 10.739$, $p = 0.004$; pair (nested within treatment) $F_{2,23} = 10.739$, $p = 0.858$). Body feathers of chicks from the same breeding colony had previously been recorded as having a $\delta^{15}N$ value of 11.6 ‰ (Bearhop 1999), which is very close to the average value of the un-fed eggs of 11.1 ‰, but considerably higher than 10.1 ‰ for eggs from fed pairs (fig. 4.5). $\delta^{13}C$ values for chick body feathers in 1996 were very different form the values found in the eggs ($\delta^{13}C$ for feathers –15.6 ‰

(Bearhop 1999); un-fed eggs –19.5 ‰, fed –20.3 ‰ (fig. 4.5)). Since whole egg samples were analysed, a high proportion of lipids were present, which are known to be isotopically depleted, with respect to $\delta^{13}C$ (Hobson 1995).

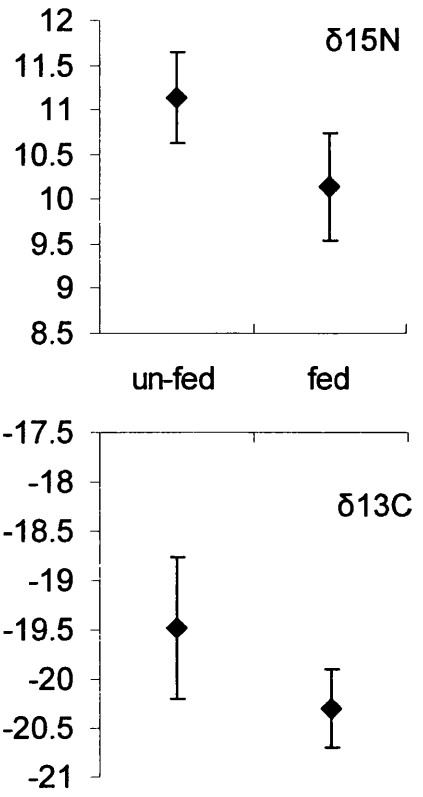

Figure 4.5: Average $\delta^{13}C$ and $\delta^{15}N$ in parts per thousand (‰) from eggs of un-fed and fed birds. Error bars denote SD of mean.

Is embryo sex related to egg composition?

Embryo sex was determined as a function of different egg components using GLMMs. None of the investigated factors could significantly explain embryo sex. However, the absolute amount of lipids as an explanatory factor was close to significance level (GLMM, with sex as dependent and pair identity as random factor: effect 0.0 ± 0.175; absolute lipids: effect –0.3129, $\chi^2 = 3.4$, $p = 0.0652$; a dispersion correction factor was included in the model and calculated as 1.017 ± 0.094). The negative sign of the effect of absolute lipids on embryo sex suggests that eggs with more lipid tended to produce more males.

4.5 Discussion

Supplying additional food prior to and during egg production did not increase egg size, clutch size or change lay date in the great skua (this study, Kalmbach *et al.* 2001). However, even though egg sequences from supplementary fed and un-fed pairs showed similar trends of size and nutrient changes, we found evidence of some subtle differences in egg composition. The differences were mostly in relation to aspects of lipid content of eggs. One such difference between un-fed and fed birds was the slower decline of total lipid amount in eggs laid by supplemented birds. Despite the decline of the absolute amount of lipids deposited into subsequent eggs, the relative lipid fraction tended to increase in later eggs. An increase in relative amount of lipids with laying order had been found in natural and extended clutches of herring gulls *Larus argentatus* (Parsons 1976). Different studies have suggested different egg components to be most vital for successful development (Carey 1996 pp. 333; Finkler *et al.* 1998; Nager *et al.* 2000). The amount of lipids in an egg is generally regarded to play a vital role for hatchling survival during the first few days (Williams 1994; Finkler *et al.* 1998). Yolk reserves often provide the energy for the chick immediately after hatching, and for several days (Starck 1998). Even though a relatively larger amount of lipids might provide a small chick with extra energy to catch up during these early days, the absolute amount could ultimately determine whether a chick can develop at all. Once an egg becomes too small, even with changes in relative nutrient proportions, it will not be able to produce a chick (Carey 1996). Later eggs of supplemented birds might therefore have had an advantage in the amount of lipids over the un-fed equivalents.

The provisioning of supplementary food affected birds differently over the season. Lay date was significantly correlated to the rate of nutrient change in eggs from both groups of birds. In general, birds which started laying later in the season, had a steeper decline of nutrients over the laying sequence. However, this seasonal pattern was more often found or was stronger in the un-fed group. For example, while in the un-fed group egg mass declined much faster over the sequence of late clutches, within the fed group, the rate of mass change did not differ for clutches initiated early or late. This indicates that particularly late pairs benefited from the additional food. In many birds, as in the skuas, late breeding is regarded as a sign of either young age or poorer individual quality, coupled with a poorer breeding capacity (Catry *et al.* 1998). It is therefore not surprising to find that supplementary food enhanced the amount of nutrients deposited in eggs by later pairs. The results from the change of relative egg composition are less clear cut. Over their laying sequence, late pairs without supplementary food increased the relative amount of both

lipids and proteins, but at the cost of decreasing the water portion. It is not clear how this affects egg quality as such. Increased relative nutrient portions might be interpreted as higher quality. A study by Hill and Schrier (in Carey 1996) concluded that the relative amount of water is the most crucial element to ensure hatchability. The removal of only 10 % rendered eggs infertile (see Carey 1996). The increased protein and lipid fractions at the cost of water loss might therefore represent a disadvantage, rather than an egg quality improvement. By contrast, in the clutches of late fed birds, the increase in relative protein content was offset by a decrease in lipids, while the water portion did not change. Egg proteins provide most of the raw material for tissue synthesis in the embryo (Hill 1993), and a large protein proportion could produce a chick which is structurally large for the size of the egg. Structurally larger size has been found to enhance survival probability to a larger extent than hatching condition, i.e. fat reserves, in chicks of the lesser black-backed gull *Larus fuscus* (Bolton *et al.* 1991). Not only the timing of laying, but also the total number of eggs produced might reflect an individual's quality. It is therefore not surprising, that the amount of lipids deposited in eggs decreased less rapidly in longer egg sequences.

It is difficult to state what determines egg quality. As outlined above, different studies have identified different egg components as being the most important to either hatchability or post-hatching survival. However, our results show that provisioning of supplementary food did enhance some aspects of egg laying capacity in the great skua. Namely, it slowed down the nutrient decline over an extended egg sequence, and enabled later birds to produce clutches with eggs of similar composition to those of earlier breeders. The stable isotopes signatures of eggs from fed and un-fed birds show fact that birds, which received supplementary food, did use part of it to provision eggs. The nitrogen signature of un-fed eggs is very similar to a previously established signature of chick feathers (Bearhop 1999). The lower $\delta^{15}N$ value for eggs from supplemented birds is indicative of diet components of a lower trophic status than the average skua diet, suggestive of catfood being used in egg production. The carbon isotope values of our study cannot be directly compared with the study of Bearhop et al. (1999), because of a high proportion of lipids in our samples. However, between our two experimental groups, the fed one had a more ^{13}C depleted signature. This is indicative of terrestrial diet components (Bearhop *et al.* 1999; Hobson 1999), which make up the main bulk of cat food. Other studies on supplementary food have shown that the type of supplement has a strong influence on the enhancement of egg production (Bolton *et al.* 1992). This is due to the fact that micro nutrients in eggs are found in different proportions than in the average ingested

food items (Houston 1997). Some of these specific nutrients are accumulated and stored in body tissues prior to the breeding season (Houston *et al.* 1995). Our results suggest that first eggs were not affected by the feeding treatment, but later eggs were to a higher degree. This could be explained if mothers rely on stored nutrients for egg production, but as they lay an increasing number of eggs, these stores become depleted and they rely more heavily on incoming food (Houston *et al.* 1995). The $\delta^{15}N$ value of eggs is expected to increase with an increasing proportion of body tissue used in egg synthesis (Hobson 1995). Preliminary results on $\delta^{15}N$ changes over the extended laying sequence of four females suggest that this might have been the case (unpubl. data). A complicated and costly procedure would need to be employed to determine which fractions of the supplementary food are transferred to the eggs (Houston et al. 1995). Our bulk analysis of isotope values in the eggs can show, however, that both lipids (mainly represented by $\delta^{13}C$ values) and proteins (represented by $\delta^{15}N$ values) from the supplementary food were used in egg synthesis.

In an analysis combining many supplementary feeding studies, Nager *et al.* (1997) found that differences between fed and un-fed groups are stronger in poor years, and less visible under favourable conditions. The year when we conducted our experiment, was one of high sandeel abundance around Shetland (Upton *et al.* 2000). This might be an important reason why, in contrast to other studies, our study showed only subtle changes in egg laying capacity due to the provisioning of extra food. While egg and clutch size and lay date were not affected, the nutrient contents over the laying sequence changed in comparison to a non-supplemented group. Isotopic analysis is a very useful tool in tracing nutrients from food to eggs (Hobson 1995; Hobson 1999). However, it has so far only been used in a few studies to trace specific components (e.g. Houston et al. 1995). While changes in egg composition might well be of an adaptive nature, they could simply reflect the availability of nutrients to the mother. In our study, lipids were the one factor, which consistently turned out as being related to the investigated aspects of egg production: feeding treatment, timing of laying and the total number of eggs laid throughout the experiment. Lipids were also the only egg component which appears to be linked to embryo sex in great skuas. How changes in egg composition affect egg and chick performance is very difficult to determine, since eggs need to be destroyed for nutrient analysis. Therefore, few studies have investigated these ecological consequences of (inferred) natural egg composition (Nager *et al.* 2000). More studies of this kind are needed to understand the link between food availability, egg laying capacity and composition, and their long term effect on chick performance.

4.6 References

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Chapter 5

High female offspring vulnerability in a bird species where females are larger

Manuscript:

E. Kalmbach, R. W. Furness and R. Griffiths. High female offspring vulnerability in a bird species where females are larger.

5.1 Abstract

In sexually size-dimorphic birds and mammals, the larger males often show increased post-natal mortality. High male vulnerability is widely assumed to be a direct consequence of larger size, but some alternative explanations have been proposed. No experimental evidence exists for elevated mortality of larger females. We investigated sex-specific offspring mortality in the great skua *Catharacta skua*. Great skuas exhibit reversed sexual size-dimorphism, with females being larger than males. Additionally to following chick survival until fledging under natural conditions, we conducted an egg-removal and cross-fostering experiment to investigate survival under harsh conditions, since harsh conditions are predicted to highlight vulnerability. The experiment also allowed us to separate parental and egg effects on chick survival. Female chicks showed a higher mortality across all treatment groups. Second chicks in a brood of two survived less well than first chicks, regardless of sex. Under experimentally created harsh conditions, mortality increased for all chicks. Poor egg quality outweighed parental effects on chick survival. While hatch weight was the single most important predictor for survival of female chicks ($b = 0.723$, $p = 0.026$), it did not predict male survival ($b = 0.045$, $p = 0.528$). Our results strongly suggest that different factors act upon the survival of differently sexed offspring in size-dimorphic species. Larger size appears costly due to increased intrinsic mortality whether males or females are larger, while the smaller sex is more affected by intra-brood competition.

5.2 Introduction

Sex-biased offspring mortality has been reported for many species of birds and mammals, including humans. In the majority of studied species, males exhibit higher mortality at some stage between conception and sexual maturity (Clutton-Brock 1985; Breitwisch 1989; Griffiths 1992; Owen-Smith 1993; Moses *et al.* 1998; Lummaa *et al.* 2001; Moss 2001). However, increased vulnerability is not an exclusively male trait, and a few studies have also reported higher female offspring mortality (De Kogel 1997; Torres & Drummond 1997; Woodroffe & Macdonald 2000). One factor that most studies have in common is that harsh circumstances highlight the 'weaker sex' effect. Some explanations focus on external factors (e.g. siblicide (Smale *et al.* 1999), competitive advantage of larger sex (Oddie 2000)), but increased vulnerability is generally thought to be due to an intrinsic sex-linked factor.

Several physiological mechanisms could explain the observed patterns. However, the mechanism cited most often refers to size differences between the sexes and an increased energy requirement that follows from larger body size (Teather & Weatherhead 1988; Clutton-Brock 1991). In times of limited resources, offspring with a higher energy demand would die first. The difference in sex-specific mortality should therefore increase with an increasing degree of sexual size dimorphism (SSD). Clutton-Brock *et al.* (1985) showed that this prediction holds true over a varying degree of sexual size dimorphism when males are larger. However, it does not appear to be equally related to SSD when females are larger. Results from studies on species with reversed sexual size dimorphism (RSSD) are ambiguous. The results of Krijgsveld *et al.* (1998) on marsh harrier *Circus aeroginosus* chicks follow the general pattern that although more costly in total, the larger sex is cheaper to rear per gram. By contrast, Newton (1978) and Torres & Drummond (1999) found that the females did not consume more energy to reach their larger fledging size. However, while in the European sparrowhawk *Accipiter nisus* no sex-specific mortality was detected (Newton 1978), nestling mortality was female-biased in the blue-footed booby *Sula nebouxii* (Torres & Drummond 1997). Final nestling size might not be the most important factor that reflects relative vulnerability throughout ontogeny. The male-biased mortality of pre- and postnatal humans, for example, is often ascribed to male heterogameity with regard to the sex chromosomes. Any deleterious mutation on the X-chromosome is more likely to be expressed by the heterogametic sex, which, in the case of mammals, are the males. In birds, the situation is reversed, as females exhibit heterogametic sex chromosomes (ZW). A third hypothesis that has been used to explain observed mortality patterns is the Immunocompetence Handicap Hypothesis (ICHH), as

proposed by Folstad & Karter (1992). The ICHH assumes a male disadvantage in immunocompetence due to a male-specific physiological trait, in particular high levels of androgens. Testosterone, for example, may have negative effects on the immune system (Grossman 1985).

A general problem in identifying the causes for sex-biased mortality is presented by the overlapping predictions these different hypotheses make. In male mammals for example, all three potential disadvantages are accumulated: they are usually larger, they are heterogametic and they exhibit higher androgen levels than females. It is therefore difficult to separate these effects. Birds have the advantage that heterogameity is separated from being male and the consequential higher levels of androgens. More importantly though, size dimorphism is reversed in a number of bird species. This is particularly useful in situations where closely related species show reversed size dimorphism patterns, which comes close to a natural experiment in which only the sizes are swapped between the sexes. Such a situation is found between gulls (*Larus* sp.), where males are the larger sex, and skuas (*Catharacta* sp.), where females are larger. Not only are gulls and skuas sister taxa (Cohen *et al.* 1997), but they show broad similarities in demography (Ratcliffe *et al.* in press), breeding (Hamer & Furness 1993; Catry *et al.* 1997), diet (Phillips *et al.* 1997), and physiology (Caldow & Furness 1993). Several studies of gulls, in particular the lesser black-backed gull *Larus fuscus*, have shown higher vulnerability and costs of sons, the larger sex (Griffiths 1992; Nager *et al.* 1999; Nager *et al.* 2000). Nager *et al.* (1999) have also shown that gull mothers skew the primary sex ratio in favour of daughters when in poor condition. In contrast, great skua *Catharacta skua* mothers overproduced sons in comparable circumstances (Kalmbach *et al.* 2001).

In the present study we investigate sex-specific offspring survival in the great skua under natural conditions, as well as under experimentally created poor conditions. Since great skuas normally lay two eggs, in the un-manipulated nests there were many broods of two. Competition between chicks within the brood is likely to have important implications for survival and can confound other factors that affect it, such as egg and parental quality. To enable us to separate effects of egg and parental quality on offspring mortality, we conducted an egg removal and cross-fostering experiment. Through egg removal, we induced females to lay six instead of the normal two eggs. Thus we created a group of poor condition parents and obtained lower quality eggs from the second half of the extended laying sequence. Fourth eggs were cross-fostered to pairs which had laid only two eggs, while sixth eggs were left with the original parents (removal group). All experimental chicks therefore hatched from poor eggs, but in the removal group chicks had the

additional disadvantage of being raised by poor condition parents. If female offspring in the great skua are more vulnerable than their brothers, we expect to see higher female mortality in the experimental (foster and removal) groups. Depending on the relative importance of parental or egg quality, such a difference should be seen in both (egg effects) or mainly in the removal group (parental effects).

5.3 Methods

The present study was carried out between May and August 2000 on Foula, Shetland (60°08'N, 2°05'W), which supports a colony of approximately 2500 breeding pairs of great skuas. Altogether we used 169 pairs of great skuas for our study, divided over three different treatment groups. Fifty-four pairs were studied in the un-manipulated group, 55 pairs belonged to the removal group and 60 clutches were originally prepared as foster nests, of which 49 were eventually needed. In the removal and foster groups we carried out daily nest checks prior to the onset of laying, un-manipulated pairs were checked every two days. In the un-manipulated group eggs were marked and measured upon finding. In a few cases both eggs of one pair were found during the same nest check, in which case it was only possible to assign laying order if both eggs hatched. Nests of un-manipulated pairs were checked every two days throughout the breeding season. During the peak of hatching, nest checks were carried out more frequently.

In order to minimise variability of adult quality amongst foster parents, we chose pairs which had laid their first eggs within a time period of 8 days (10 to 17 May) in the first half of the laying season. Similarly, for the group of removal pairs, we only chose pairs which had initiated laying within a 10 day period (10 to 19 May). Eggs of foster parents were vigorously shaken and painted with clear varnish after clutch completion, in order to prevent development of embryos. During the painting procedure, dummy eggs were placed into the nest. For cross-fostering, one of the original eggs was replaced by an experimental egg, so that foster parents were still left with two eggs for incubation. Nest checks were kept to a minimum until hatching time, when relevant nests were checked daily.

In the removal group, daily checks were carried out and any newly laid egg was removed upon finding. Fourth eggs laid by these females were put into nests of foster parents. We attempted to keep egg quality of fourth eggs as similar as possible by only cross-fostering those fourth eggs, which had been laid between 20 and 31 of May (12.0 days ± 2.17 after first eggs were laid). Of 46 pairs we continued egg removal until a sixth egg was laid, which we left in the nests of removal parents. Within two days after laying,

we added a dummy egg to the nest, in order to prevent females from laying a further egg. However, 15 females produced a seventh egg despite the dummy egg or just before it was added. We assume that females who laid a seventh egg were in comparatively better condition at the time when they produced the sixth egg, than those females who did not produce a seventh egg. We therefore believe that rather than increasing the variability of parental condition within the removal group, the production of these seventh eggs might have reduced variability between females. In only four of these nests both eggs hatched, in three neither hatched, and of the remaining eight, one chick came from a seventh egg, while the others hatched from the sixth. Because of our aim to prevent competition within broods, we carried out the survival analyses for experimental chicks with and without the inclusion of the four nests which had hatched two chicks. None of the conclusions were affected by the exclusion of those nests. Therefore the results we present here include all chicks.

In all treatment groups chicks were measured and marked by painting different combinations of toenails on the day of hatching or the following day. A 50 µl blood sample was taken when chicks were marked. The blood samples were later used for molecular sexing, following the method described by Griffiths *et al.* (1998), with the alterations given in Kalmbach *et al.* (2001). At around 10 days of age, chicks were ringed with an individually numbered British Trust for Ornithology ring. Survival of all chicks was followed by nest and territory checks every two to three days. Parental behaviour was included in decisions about disappearance / death of nestlings. Chicks were classified as 'fledglings' when they reached the age of 38 days, as chicks start to fly at around 38 to 44 days old, and survival from 38 to 44 days is generally high.

Chick survival was analysed by fitting logistic regressions. Originally, we included all interactions of the respective main factors, and then followed backward elimination of factors that contributed least in explaining the observed variance, starting with the highest order interactions. Significance of explanatory variables was determined by the increase in total variance after removal of the variable. In the final models, only significant interactions are included. Sex ratios are given as proportion of males. Statistical analyses were performed using SPSS for Windows, version 10.0. Data are presented as means ± s.e. with n denoting sample size. All statistical tests are two-tailed and the significance level was set at 5%.

5.4 Results

Un-manipulated broods

Overall hatching success was 75 % (n = 108). The sex ratio at hatching was 0.53 (n = 81), which was not different from a 1:1 binomial distribution (binomial test p = 0.734). Of the hatchlings, 65 % survived to fledging (at 38 days). The fledging sex ratio was slightly biased in favour of males with 0.61, but was not different from unity (binomial test p = 0.161). Female chicks had a lower survival probability from hatching to fledging than male chicks (survival ♂: 0.76, n = 43; ♀: 0.54, n = 38), but this difference was not statistically significant (logistic regression, dependent: survival; sex χ^2 = 3.301, df = 1, p = 0.069, n = 81; Fig. 5.1).

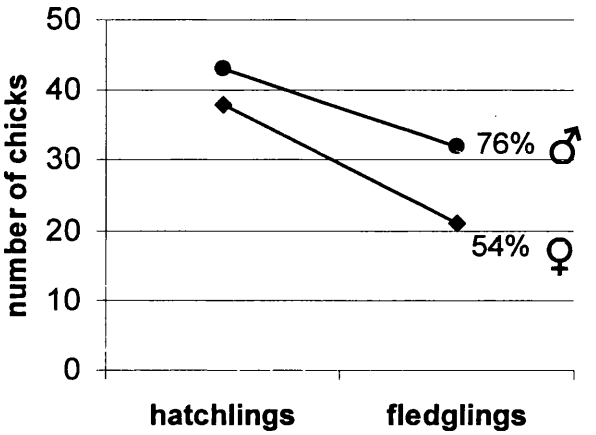

Figure 5.1: Survival of male and female chicks from hatching to fledging (χ^2 = 3.301, df = 1, p = 0.069, ♂n = 43, ♀n = 38).

However, great skuas usually lay a clutch of two eggs, and 59 % (n = 51) of nests hatched two chicks. Competition within broods may have an influence on chick survival. As well as the brood position, the condition of chicks at hatching may also play an important role. We therefore included chick position and hatchling weight in addition to sex in the model to predict chick survival. When corrected for hatchling weight and chick position, the survival difference between male and female chicks was significant (Tab. 5.1). Hatching in second position was a disadvantage for both sexes (Fig. 5.2). Females also showed poor survival when being a single chick, while male single chicks survived almost as well as when first chick. Sibling sex was originally included as a factor in the full model, but did not have a significant effect on chick survival (χ^2 = 0.493, df = 1, p = 0.483). Hatchling weight did not affect survival, but it had a different effect for chicks from the three positions (Tab. 5.1). For chicks in the second position, increasing hatch weight tended to enhance survival probabilities (b = 0.055, p = 0.275). The trend for first

and single chicks was in the opposite direction (b = -0.093, p = 0.142). These results show that chick position within a brood has a significant effect on chick survival, on its own as well as in combination with hatchling weight. The poor survival of second chicks could be due to several factors. Intra-brood competition and egg quality are two possible reasons. Second chicks hatch from second laid eggs, which might be of poorer quality than first eggs. Also, hatching slightly later will accentuate a hierarchy within the brood. The fact that hatch weight plays a different role for chicks in different positions suggests that hatch weight by itself might only be crucial under harsh conditions. In the following experiment we therefore looked at the survival of chicks from eggs of experimentally induced poor quality reared by parents of differing condition.

Table 5.1: Results of a logistic regression of chick survival in un-manipulated broods. Only significant interactions are presented.

	χ^2	df	p
Sex	6.549	1	0.010
Hatchling weight	0.556	1	0.456
Chick position	6.850	2	0.033
Hatchling weight * Chick position	6.033	2	0.049
Model (n = 69)	13.768	6	0.032

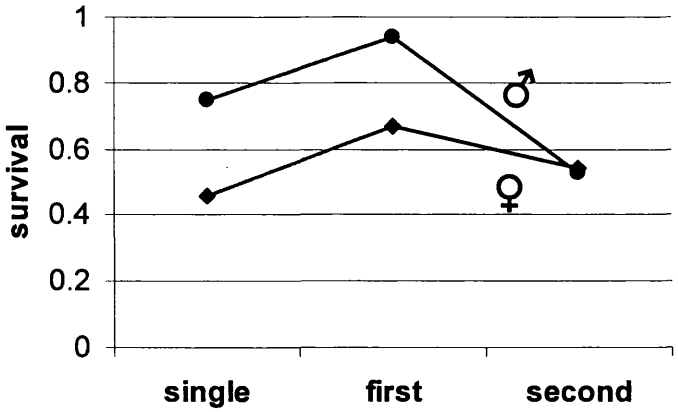

Figure 5.2: Survival of male and female chicks dependent on position within brood (first or second chick in brood of two and only chick in single chick broods; ♂n: single = 8, 1st = 18, 2nd = 15; ♀n: single = 13, 1st = 12, 2nd = 11; statistical results see Table 1).

Experimental conditions

Of the 49 fourth eggs that were cross-fostered, 27 hatched chicks (55 %) and 3 chicks died at hatching. Of the 19 remaining eggs, 2 disappeared and 17 were addled. Hatching sex ratio was 0.52, which equals a 1:1 ratio (binomial test p =1.0). With an average survival probability of 0.52 from hatching to fledging, survival in this group was not significantly lower than under natural conditions (χ^2 = 1.586, df = 1, p = 0.208). Male and female chicks did not have significantly different survival probabilities, but as under natural conditions the trend was that female chicks survived less well (♂0.57, n = 14, ♀0.46, n = 13; χ^2 = 0.326, df = 1, p = 0.568).

In the group of sixth and seventh eggs that were incubated by their natural parents, 29 of 61 eggs gave rise to hatchlings (48 %). With a hatchling ratio of 0.66 the removal group had a tendency towards a male bias (binomial test p = 0.136). Survival of these hatchlings to fledging age was 0.48, not significantly lower than under natural conditions (χ^2 = 2.640, df = 1, p = 0.104), and very similar to the cross-fostered chicks (χ^2 = 0.072, df = 1, p = 0.789). Again, females had a non-significant tendency of lower survival (♂0.53, n = 19, ♀0.40, n = 10; χ^2 = 0.419, df = 1, p = 0.518).

In both experimental groups hatching success and survival were very similar. Likewise, hatchling weight was not different between the experimental groups, but was very different from un-manipulated chicks (control: 65.6g ± 6.8, n = 69, foster: 58.5g ± 5.4, n = 25, removal: 56.8g ± 4.3, n = 28; ANOVA: $F_{2,121}$ = 27.213, p < 0.001, post-hoc Scheffé: foster-removal p = 0.595, control-foster / removal p < 0.001). We therefore pool chicks from the two experimental groups in order to investigate the effect of hatchling weight on survival. When corrected for hatch weight, experimental chicks had a significantly lower survival probability than control chicks, and for chicks from all treatment groups together, lighter chicks survived less well than heavier chicks (hatch weight coefficient b = 0.062, p = 0.025). However, hatch weight affected survival differently for control and experimental chicks (logistic regression, dependent: survival; treatment χ^2 = 4.295, df = 1, p = 0.038; hatch weight χ^2 = 4.723, df = 1, p = 0.030; treatment*hatch weight χ^2 = 3.975, df = 1, p = 0.046). While on average, hatch weight did not affect chick survival for control chicks (see above), in the experimental group there was a strong positive relationship between hatch weight and survival (logistic regression, dependent: survival; hatch weight χ^2 = 6.101, df = 1, p = 0.014; b = 0.151). However, this relationship differs significantly between the sexes (Tab. 5.2). While male survival was equally poor across the whole range of hatch weights (♂ hatch weight coefficient b = 0.045

± 0.071, p = 0.528), very light females had a much lower survival probability than heavier ones (♀ hatch weight coefficient b = 0.723 ± 0.325, p = 0.026; Fig. 5.3).

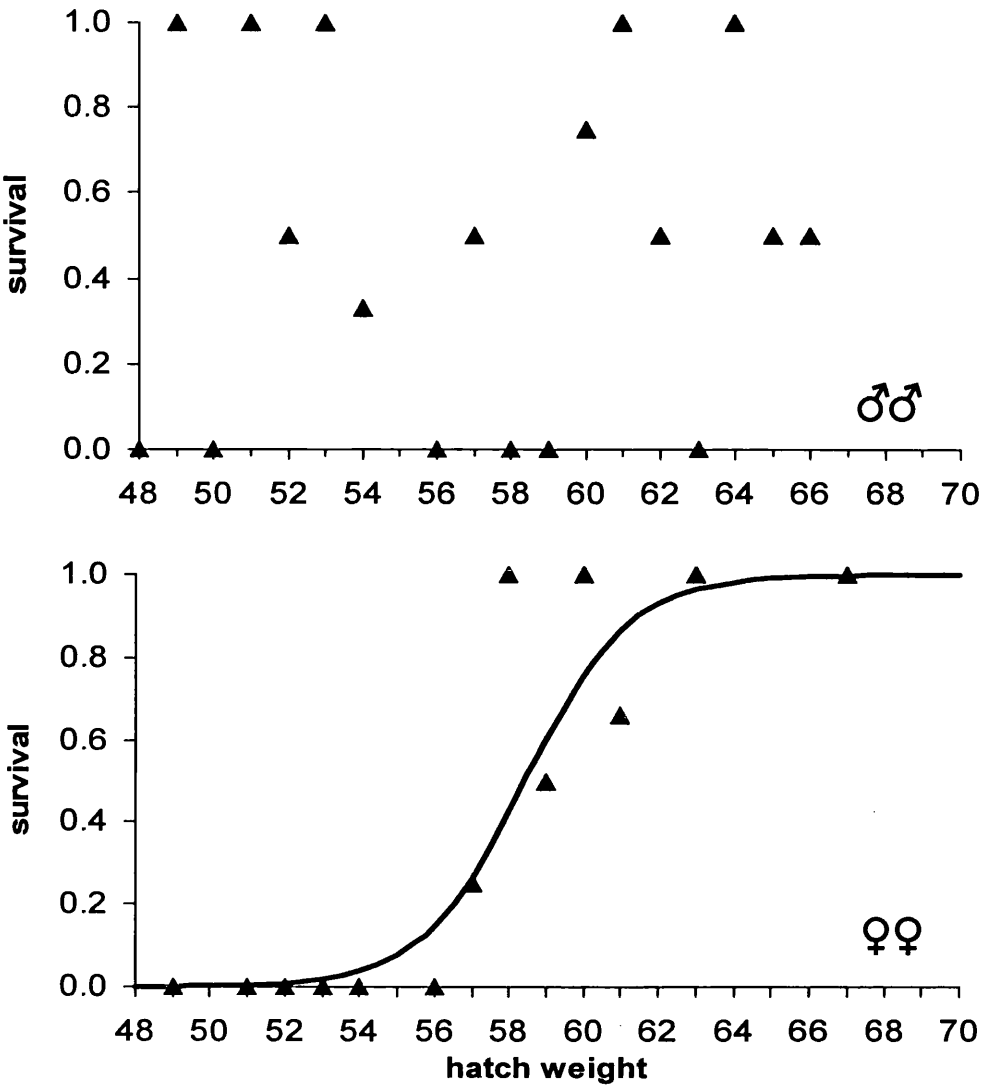

Figure 5.3: Experimental chick survival dependent on hatch weight of chicks for male and female chicks. Triangles represent the actual data, the line in b) represents the prediction of a logistic regression of survival over hatch weight for females (regression $\chi^2 = 14.394$, df = 1, $p < 0.001$, $r^2_{Nagelkerke} = 0.648$).

Table 5.2: Results of a logistic regression of experimental chicks' survival in relation to sex and hatch weight. A factor for treatment (foster or removal, see text) was originally included, but did not explain a significant part of the variance.

	χ^2	df	p
Sex	10.794	1	0.001
Hatchling weight	15.952	1	0.000
Hatchling weight * Sex	10.597	1	0.001
Model (n = 53)	15.395	3	0.002

5.5 Discussion

In the reversed sexually size-dimorphic great skua, female offspring exhibit higher vulnerability than male offspring. Female-biased mortality occurred both in un-manipulated broods and under experimentally created poor conditions. The observed mortality pattern is opposite to the pattern found in the closely related lesser black-backed gull, in which the sons suffer elevated mortality (Griffiths 1992; Bradbury & Griffiths 1999). Importantly, in the gulls the males are the larger sex. Our results combined with the studies of gulls give strong support to the hypothesis that size *per se* makes the larger sex more vulnerable in species with sexual size dimorphism.

Theory also predicts that under harsh conditions the more vulnerable sex should show a greater reduction in survival than the resilient one (Sheldon *et al.* 1998). In natural great skua broods of two, second chicks had a survival disadvantage compared to first chicks. Contrary to the prediction however, hatching second resulted in an equally steep decline for the survival of sons and daughters. This high male mortality in second position is likely to be due to competition within broods. The size hierarchy within a brood is generally increased by asynchronous hatching. As males are smaller, being second would compromise the males' competitive ability more than that of females. Other studies have also found a competitive disadvantage of the smaller sex within broods (Bortolotti 1986; Oddie 2000). Daughters' survival, by comparison, is relatively low in all positions within a brood, being first, second or even single chick. This points towards an intrinsic mechanism, which results in a higher baseline vulnerability for female chicks. Such a mechanism could be based on sex-specific growth rates, if female chicks had to achieve a higher initial growth rate in order to develop normally throughout the nestling phase

(Gebhardt-Henrich & Richner 1998). Female great skua chicks do not hatch heavier than male chicks, but at the end of the nestling phase they fledge at a higher mass than males (chapter 6). This means their development pattern must be different from that of males. Mortality occurred mainly in the first ten days after hatching, but not exclusively. However, this does not exclude the possibility that processes in the early days of life are deterministic for later survival. Several studies have shown that conditions during early development have consequences on later survival (Elo & Preston 1992; De Kogel 1997; Lindström 1999). On the whole, hatchling weight did not have a significant effect on chick survival under natural conditions. However, the trend of a positive correlation between hatch weight and survival for chicks in second position suggests that under harsh conditions, hatch weight might well become a crucial factor.

Results from the experimental chicks confirm that hatchling weight can play a crucial role for survival. Furthermore, they provide strong evidence that male and female offspring in the great skua are sensitive to different variables. Compared to un-manipulated broods, offspring survival was reduced in the experimental groups for both sexes of offspring, with consistently lower female survival. Survival was not significantly reduced in the group that had the additional disadvantage of being raised by poor condition parents compared to the ones raised by foster parents. This strongly indicates that egg quality was a more important determinant of offspring survival than parental condition. At first sight, this result might seem somewhat surprising, since it has been shown that increased egg production effort results in lower offspring rearing capacity in lesser black-backed gulls (Monaghan *et al.* 1998). However, in the study of Monaghan *et al.* (1998), parents raised a control clutch after having laid one additional egg. By contrast, gull chicks hatching from poor quality eggs but being raised by control parents also showed increased mortality, particularly the male chicks (Nager *et al.* 1999). While parental condition evidently affects rearing ability, it appears that once eggs fall below a certain quality threshold, egg effects become so strong that they outweigh differences in parental quality.

Since intra-brood competition had been removed by having chicks reared singly, an intrinsic factor is most likely to explain the mortality pattern we observed in the experimental group. For daughters but not for sons, hatchling weight became a crucial factor additionally to the overall reduced survival in the experimental group. Hatchling weight is tightly correlated to egg mass and reflects to a certain extent the amount of resources a chick possesses during the first days of life (Furness 1983; Carey 1996). Different metabolic requirements of male and female chicks during the initial growth phase could explain different sensitivities to low hatch weight. Other studies of size dimorphic

species have found that the larger sex is more restricted in its growth pattern (Lindén 1981). That is, the larger sex has not got an equal scope to vary growth or development in response to resource availability. It appears that in great skuas, below a certain weight irreversible damage occurs in female hatchlings, which even under good rearing conditions cannot be repaired.

Sex-specific offspring performance has ramifications for many aspects of life history, including the theory of adaptive sex allocation. According to the hypothesis by Trivers & Willard (1973), parents should skew their offspring sex ratio in favour of the more beneficial sex. For the present study, that would mean sons are more beneficial in circumstances when the hatchling weight is likely to be very low. In a previous study we found that great skuas skew the primary sex ratio in favour of sons at the end of an experimentally extended laying sequence when eggs are considerably smaller than in normal clutches (Kalmbach *et al.* 2001). Interestingly, we found the same sex ratio skew replicated in the present study. When calculating the sex ratio in the same way as in the previous study, i.e. taking the last viable egg of each female, the sex ratio of last eggs was again significantly male biased (sex ratio = 0.73, n = 26, binomial test $p = 0.029$). In combination, these results suggest a possible adaptive nature of the observed sex ratio in last eggs.

Our study gives strong evidence that different factors can act upon survival of male and female offspring in size-dimorphic species. While the smaller sex suffers more from intra-brood competition, the larger one appears to pay a cost for its size in the form of increased vulnerability, particularly when resources during early development are limited. The fact that hatch weight plays a crucial role suggests that circumstances during early development are deterministic for these costs and cannot be offset by good parental care. Our study suggests that the sex ratio skew observed in this species (Kalmbach *et al.* 2001) might be a response to this sex-specific vulnerability.

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Chapter 6

Sex-specific growth patterns in the great skua: daughters are struggling

Manuscript:

E. Kalmbach, R. W. Furness and R. Griffiths. Sex-specific growth patterns in the great skua: daughters are struggling.

6.1 Abstract

Higher mortality of the larger sex has been reported in several size-dimorphic birds. It is generally assumed that this is due to a higher energy demand of the larger sex increasing susceptibility to food shortage. On the other hand it has been suggested that sex-specific growth rates might act to reduce discrepancies in energy demands of differently sized chicks. In order to test these ideas, we studied growth of male and female chicks in a reversed size-dimorphic seabird, the great skua *Catharacta skua*, for which a higher mortality rate of female chicks has been shown. We fitted logistic growth curves to the data obtained for mass, tarsus and wing increment. We used the fitted parameters to compare growth between sexes, timing of growth of mass, wing and tarsus within sexes, and the influence of hatching condition on growth. At the end of the fledgling period, great skua chicks exhibited the same degree of size dimorphism as is found in adults, although neither sex had reached final adult size. Females grew significantly longer wings and were heavier, but did not take longer to complete development except in feather growth. Females had higher mass growth rates particularly in the second half of the nestling period. Increased investment into mass increase by females was set off by a delay of feather growth. Low hatching condition affected the age at which females reached maximum mass gain, but did not affect male growth. In both sexes slow growth was connected to a low survival probability. It appears that females face higher energetic demands during growth, which result in a higher vulnerability. However, vulnerability appears to be connected to circumstances during early development, a time at which the dimorphism is not yet apparent in terms of size or weight. We propose that nutritional constraints during early life negatively affect the development of structures or processes that are necessary to sustain high growth rates later. We found no evidence that growth rates are adapted in order to reduce differences in sex-specific offspring cost in size dimorphic species.

6.2 Introduction

In several size-dimorphic bird species it has been shown that the larger sex is more vulnerable to poor rearing conditions than the smaller one, which is often reflected in a higher mortality rate (Røskaft & Slagsvold 1985; Teather & Weatherhead 1988; Griffiths 1992). As these studies almost exclusively included species with larger males, some authors suggested that male-specific aspects of phenotype other than size were mainly responsible for the increased vulnerability (Folstad & Karter 1992; Potti & Merino 1996). However, recent studies on sexually size dimorphic birds with larger females have strengthened the argument that sex-specific vulnerability is indeed most likely to be connected to larger size *per se* (Torres & Drummond 1997; Kalmbach *et al. in prep*). What is still not clear, is why the larger sex is more vulnerable. Generally, it is assumed that the larger sex needs more energy to grow to its larger size, and this higher energy requirement renders the chicks more vulnerable to a shortage of resources (Clutton-Brock 1991; Griffiths 1992; Teather & Weatherhead 1994). However, sex-specific growth rates might act to reduce the differences in energy requirements inferred from size alone. Sex-specific growth could be one reason why studies on sexually size-dimorphic species have rarely found results in support of Fisher's (1930) sex ratio predictions (Richter 1983,Gowaty 1991, but see Richner 1991).

Reaching different sizes at fledging can be achieved by relative variation of different aspects of growth between the sexes (Richner 1991). In short, given a certain size that is to be reached, the time taken to complete development and the daily rate of increment are the two main factors that determine the shape of growth. A particular form of growth exhibited by one sex is followed by distinct consequences for energy requirement in relation to the other sex. Richter (1983) and Rofstad (1986) for example argued that the smaller sex grows as rapidly as the larger one, resulting in earlier development, which would render the chick energetically more expensive than expected simply on the basis of its final size. By contrast, Richner (1991) showed that equal growth rates of the larger and smaller sex only occur in exceptional cases, whereas in the majority of sexually size-dimorphic species the larger chick grows at a higher absolute rate than the smaller one. Thus, growth patterns can tell us more about the energy needs during development than the extent of size dimorphism at the time of fledging, and might provide insight into the mechanism for size-dependent offspring vulnerability.

Several studies of size-dimorphic species have compared the rate of development of different body parts between the sexes. Generally, the smaller sex matures more quickly and reaches a higher proportion of the adult size by the time of fledging than the larger one

(reviewd in Teather & Weatherhead 1994). Much less attention has been paid to sex-specific patterns of timing of growth of different tissues and the possible link with sex-specific vulnerability. The relative timing of the development of body parts within one sex could provide important information on energetic trade-offs and constraints during growth. Across a wide range of species it appears that growth is maximised to physiological limits for a given size, and this also seems to hold true for size-dimorphic sexes within a single species (Ricklefs 1979; Teather & Weatherhead 1994). If growth is at its limits in the smaller sex, we would expect that an increase of the investment into any aspect of growth in the larger sex would have negative implications for the growth of other tissues. Feather development for example is a highly demanding process in adult birds, and probably equally so in growing chicks (Murphy 1996). Richter (1983) found some evidence that energetic demands for feather growth interact antagonistically with the demands of mass increase in chicks. If one sex is more tightly constrained in its growth, we would expect such trade-offs to be more pronounced.

In the present study, we investigated sex-specific growth of nestlings of the great skua, *Catharacta skua*. Adult great skuas exhibit female-biased sexual size-dimorphism and in a recent study it was shown that female chicks exhibit higher mortality than male chicks (Kalmbach *et al. in prep*). Survival of daughters was particularly poor when they hatched with very low body mass, while sons' survival was not related to mass at hatching. This suggests that male and female chicks experience different demands during development, which is likely to be related to the size dimorphism found in adult birds. In addition to presenting the growth patterns of male and female chicks from natural broods, we used chicks from an egg removal experiment (Kalmbach *et al. in prep*) in order to investigate sex-specific effects of low hatch weight on development.

6.3 Methods

The study was carried out between May and August 2000 on the island of Foula, Shetland (60°08'N,2°05'W). We followed the growth of 81 chicks from 51 un-manipulated great skua broods plus 56 chicks from an egg removal and cross-fostering experiment (see Kalmbach *et al. in prep*). In the un-manipulated group, 21 nests had single-chick broods and 30 hatched two chicks. There was no effect of hatching order on chick sex and no combination of offspring sexes in a two-chick brood was more frequent than expected by chance. Brood size did not affect growth parameters (we tested all growth parameters for differences between one and two chick broods using t-tests; all tests were non-significant, $0.110 \leq p \leq 0.995$). We therefore include each chick as an individual data point for the

comparison of growth of male and female chicks. Great skuas normally lay clutches of two eggs. In 55 experimental pairs, laying of additional eggs was induced by continuous egg removal until the production of a sixth egg. The sixth egg was then left with the parents to be raised. Several pairs produced a seventh egg, which was also left, resulting in four experimental broods with two hatchlings. The fourth eggs of the extended laying sequences were incubated and raised singly by foster parents, who themselves had only laid the normal clutch of two eggs. All chicks hatching from fourth, sixth and seventh eggs are referred to as experimental chicks, from fourth eggs only as foster chicks, and from sixth and seventh eggs as removal chicks.

Nest checks were carried out every two days, and more frequently around the expected time of hatching. At hatching or on the following day, chicks were marked by painting individual combinations of toenails until there were old enough to be ringed with a metal ring. A small blood sample (ca. 50 µl) was taken from each chick for molecular sexing. Chicks were measured every two to three days, at each occasion taking a measure of their body mass, wing and tarsus length. Body condition at hatching was calculated as a body mass index (BMI) correcting mass for size. As a measure of size we used wing*tarsus length, which provided a better fit than each of the two parameters on their own. Chicks were defined as fledglings at 38 days old, at about which time they start flying.

To describe the growth patterns in numerical terms, logistic growth curves of the form

$$y = A / 1 + e^{(-k*(age - ti)}$$

were fitted to the data for body mass, wing and tarsus with age in days after hatching. Parameters estimated by the curve fitting were A = asymptotic size, k = growth constant which is inversely proportional to the time taken between hatching and fledging, and t_i = inflection point, which represents the time of maximum growth (fig. 6.1). Firstly, in order to describe the normal growth of male and female chicks, only un-manipulated chicks that had reached fledging age were included. All three parameters fitted by the logistic growth curve are interdependent. For a poorly growing chick, particularly if it died or disappeared before fledging, this can result in highly unrealistic values. The estimate of the asymptotic size is likely to be too low, but would be reached at an earlier time, thereby shifting k and t_i closer to values obtained from healthy growth. In order to gain meaningful values for comparison amongst all chicks, including un-fledged and experimental chicks, the asymptotic value was fixed at the value obtained for normal growth when calculating k and t_i for all other chicks. This procedure might produce slightly artificial values for k and t_i,

due to a possible overestimation of the final size that would have been reached by poorly growing chicks. On the other hand, un-fledged chicks never did reach an asymptotic size, and therefore any estimated value for A directly from the data is also likely to include an error. The calculation of k and t_i with the fixed value for A provides us with values that are directly comparable for all chicks, similar to correcting growth parameters for final size. For all calculations only chicks which were alive at least until 16 days after hatching were included. Instantaneous growth rates, i.e. the daily mass increment at a given age, were calculated for ages t = 0, 5, 10...40 days for control male and female chicks, as

$$\text{growth rate (t)} = k\, y_t\, (1 - y_t A)$$

with y_t = mass at age t (see Richner 1991). As this calculation relies on an estimate of A for every chick, we only calculated the instantaneous growth rate from the above formula for fledged control chicks. As an estimate of the instantaneous mass growth rate over the time of approximately linear growth (taken to be between 10 and 25 days post-hatching) for experimental or non-fledged chicks, we used the coefficient of a linear regression of mass over age for each individual. This value is referred to as linear growth rate.

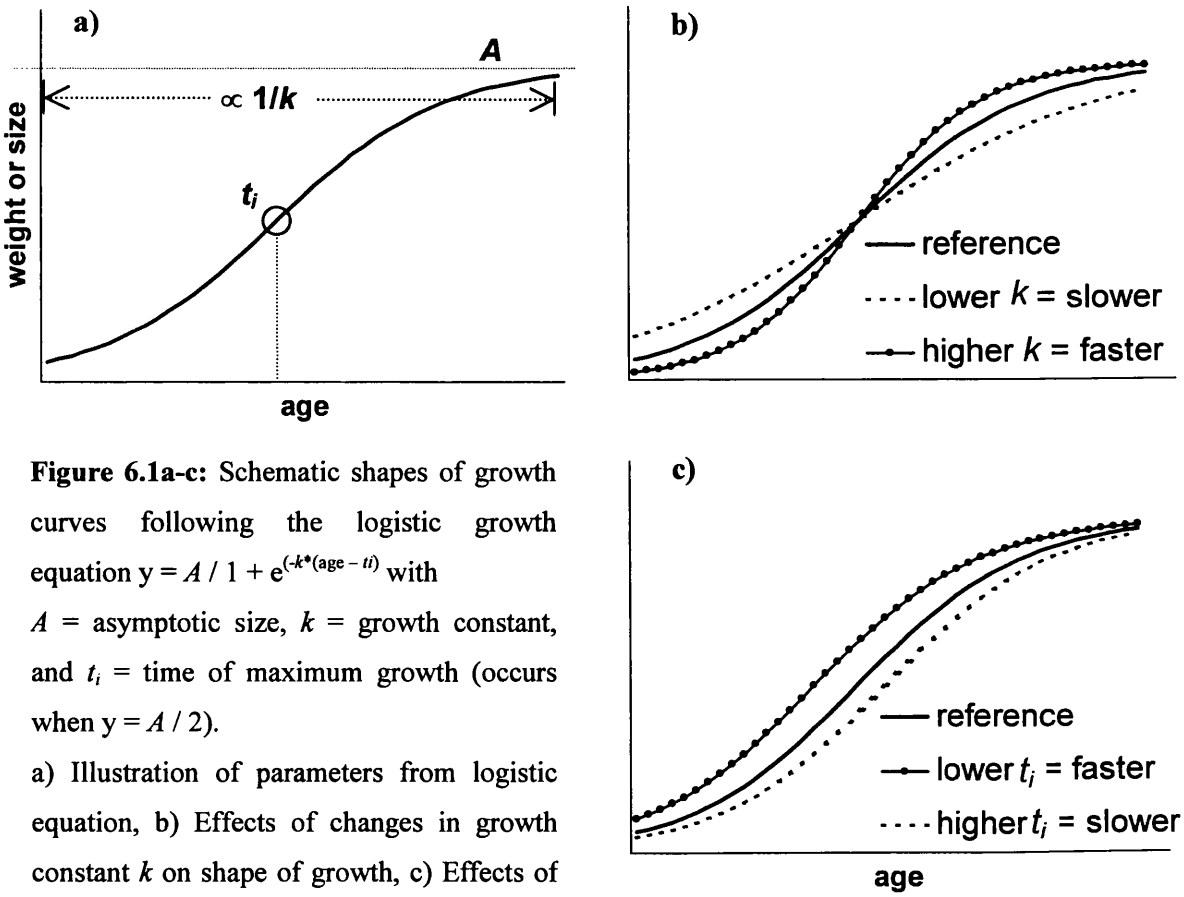

Figure 6.1a-c: Schematic shapes of growth curves following the logistic growth equation $y = A / 1 + e^{(-k*(age - ti))}$ with

A = asymptotic size, k = growth constant, and t_i = time of maximum growth (occurs when $y = A / 2$).

a) Illustration of parameters from logistic equation, b) Effects of changes in growth constant k on shape of growth, c) Effects of changes in time of maximum growth t_i on shape of growth.

Differences in instantaneous growth rates between males and females were analysed using a repeated measures ANOVA design with sex as between-subject factor. Other growth parameters obtained from the logistic growth curves were compared using t-tests for control chicks and ANOVA for comparison between treatment groups. Significances were corrected using the sequential Bonferroni procedure (Sokal & Rohlf 1995). For the analysis of mass growth in relation to development of other body parts, hatching condition and chick sex an ANCOVA design was used. To investigate the relationship between growth and survival, we used a logistic regression with stepwise backward elimination of the least significant variable, starting with interaction terms. Statistical analysis was carried out using SPSS for Windows version 10.0.

For comparative use, we also present data on sizes of adult male and female great skuas, which were caught and ringed during the years 1999 to 2001. All adult birds were caught during incubation. Weights of birds which were part of an experiment were not included.

6.4 Results

Normal growth of male and female chicks (control chicks)

The growth of body mass, tarsus length and wing length of great skua chicks can be described by logistic growth curves (fig. 6.2). For all three measurements, the logistic growth curve provides a very good fit with high r-squared values (fig. 6.2). The tarsus shows the most rapid increase directly after hatching, and reaches its final size first, while the wings grow more slowly and have not reached their asymptotic size by the time of fledging. Mass gain reaches its maximum at a time between maximum skeletal and wing growth. The growth pattern of the tarsus is not different for male and female chicks, but mass and wing development show significant differences between the sexes. Even though at hatching there is no mass difference, between 15 to 20 days post-hatching female chicks start being heavier and around fledging they reach an asymptotic mass which is 1.14 times that of males (tab. 6.1). Females also grow longer wings than males (1.05 times), but males grow their wings slightly earlier during the nestling phase than females. Until an age of about 40 days, female wings are on average shorter than male wings, and only around fledging does the wing length of females overtake that of males. For the growth constant k we did not find significant differences between the sexes for mass, wing or tarsus, which indicates that males and females needed the same length of time to reach fledgling size.

Averaged over the whole nestling period, females had a higher instantaneous growth rate of mass than males (fig. 6.3; result of rmANOVA of growth rates at ages 0, 5, 10...40 for between-subject factor sex: $F_{1,49} = 10.294$, $p = 0.012$). However, the differences in growth rate were only pronounced in the second half of the nestling period. Relative to adult size (see appendix 6-A), close to the time of fledging female chicks had reached 86 % of mass, 100 % of tarsus length and 84 % of wing length of adult size. At the same time males had reached very similar adult proportions, with 84 % of mass, 100 % of tarsus and 81 % of the wing length.

Table 6.1: Average growth parameters for body mass, tarsus and wing length for male and female chicks as derived from logistic curve fitting. A = asymptotic size, k = growth constant, t_i = inflection point (days). Only chicks measured to at least 35 days and alive at age 42 were included. (asymptotic size is not adult size, but the asymptotic value of the nestling as determined from curve fitting). An asterix in the last column marks a significant result after sequential Bonferroni correction was applied to the multiple t-test results.

	males	**females**	**t-test t / p**	**Corr. sign.**
Body mass (g)	n = 31	n = 20	t_{49}	
A	1085 ± 209	1231 ± 110	-2.888 / 0.006	*
k	0.154 ± 0.014	0.146 ± 0.017	1.810 / 0.076	
t_i	17.3 ± 1.9	18.5 ± 2.2	-2.062 / 0.045	
Tarsus length (mm)	n = 31	n = 20	t_{49} ($t_{27.7}$ for A)	
A	70.4 ± 1.4	71.3 ± 2.4	-1.589 / 0.123	
k	0.130 ± 0.018	0.131 ± 0.019	-0.086 / 0.932	
t_i	5.6 ± 1.2	5.9 ± 1.2	-1.069 / 0.290	
Wing length (mm)	n = 29	n = 19	t_{47} ($t_{26.8}$ for t_i)	
A	342 ± 12.2	359 ± 16.2	-4.208 / 0.000	*
k	0.120 ± 0.010	0.113 ± 0.008	2.407 / 0.020	
t_i	24.9 ± 1.3	26.9 ± 2.4	-3.739 / 0.002	*

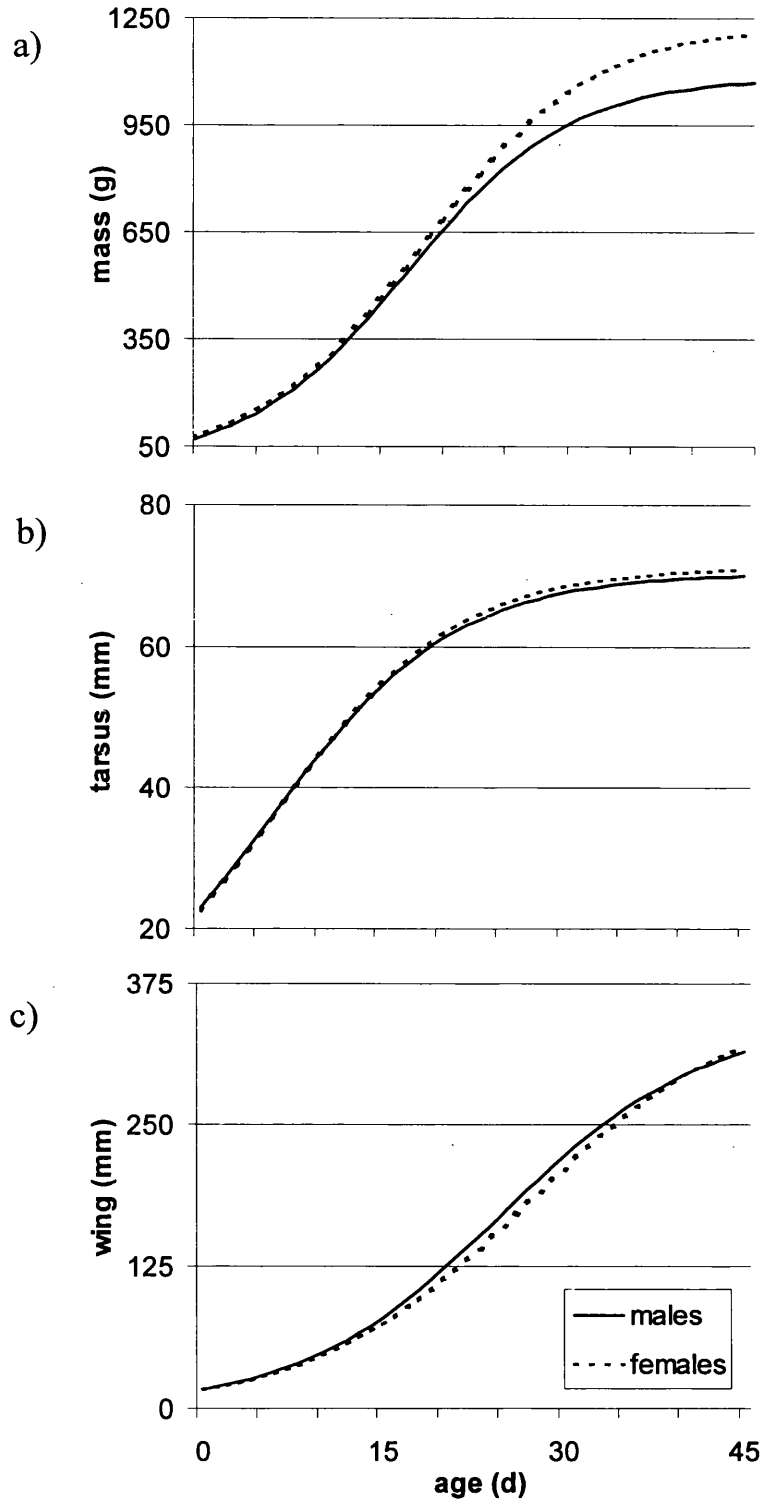

Figure 6.2a-c: Body mass, tarsus length and wing length growth of male and female chicks. Only chicks measured to at least 35 days and alive to fledging age at around 42 days were included. Average r^2 are mass: 0.992 ± 0.007 (51), tarsus: 0.994 ± 0.004(50), and wing: 0.997 ± 0.006 (49).

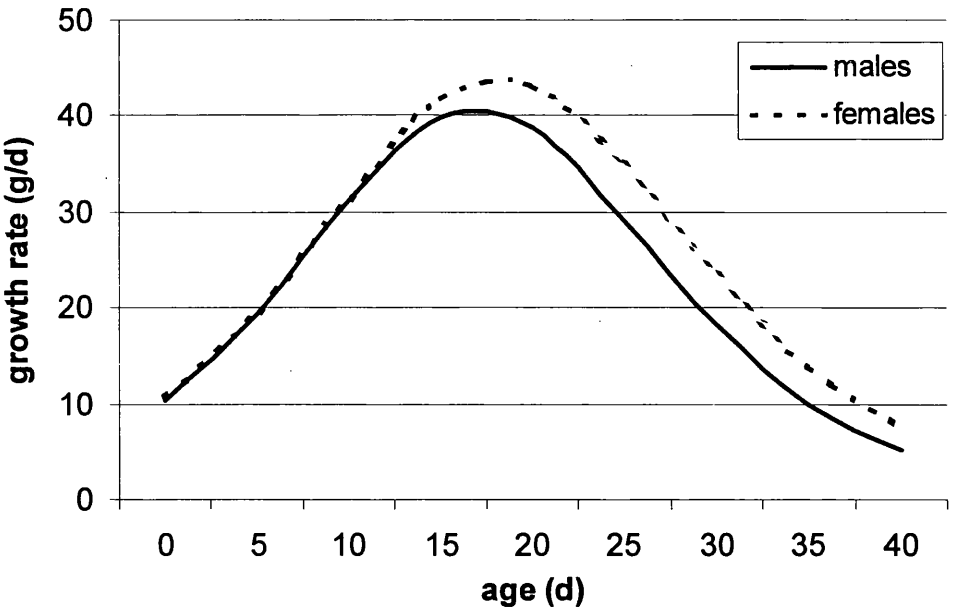

Figure 6.3: Instantaneous growth rate of mass for male and female chicks over age. Females had higher growth rates than males in the second half of the nestling period (result of rmANOVA of growth rates for between-subject factor sex for ages 0, 5, 10, 15, 20: $F_{1,49} = 0.893$, $p = 0.349$; ages 20, 25, 30, 35, 40: $F_{1,49} = 10.294$, $p = 0.002$).

Sex differences in timing of growth (control chicks)

Clearly, the growth of different parts of the body are expected to be highly correlated. A slow growing chick will be slow in all aspects of its development. However, the exact timing of maximum growth of different body parts might play a crucial role in resource availability to different tissues. As a measure of timing of development we use t_i (time of maximum growth, given in days after hatching) of the different parameters (mass, tarsus, wing). As expected, the time of maximum growth for all three parameters was highly correlated ($r(t_{i\text{-mass}} - t_{i\text{-wing}}) = 0.791$, $p < 0.001$; $r(t_{i\text{-mass}} - t_{i\text{-tarsus}}) = 0.730$, $p < 0.001$; $r(t_{i\text{-wing}} - t_{i\text{-tarsus}}) = 0.423$, $p = 0.002$). We have already shown that the maximum wing growth for females occurred later in the nestling period than for males (tab. 6.1). Additionally, there was a sex difference in the timing of maximum feather growth in relation to the maximum rate of mass increase. The regression slope of $t_{i\text{-wing}}$ on $t_{i\text{-mass}}$ is twice as steep for females as for males (fig. 6.4). Hence, amongst the fastest growing chicks, sons and daughters which reach maximum weight gain at the same time after hatching will also reach maximum feather growth at the same time. However, when reaching $t_{i\text{-mass}}$ progressively later, female chicks showed an increasing time gap until

reaching $t_{i\text{-wing}}$ compared to males. This suggests a higher trade-off between investment into mass gain and feather growth for females. A similar sex-linked trade-off could not be detected between mass and tarsus (dependent: $t_{i\text{-tarsus}}$, factor: sex* $t_{i\text{-mass}}$ $F_{1,49} = 0.122$, $p = 0.729$) or tarsus and wing (dependent: $t_{i\text{-wing}}$, factor: sex* $t_{i\text{-tarsus}}$ $F_{1,49} = 0.116$, $p = 0.736$).

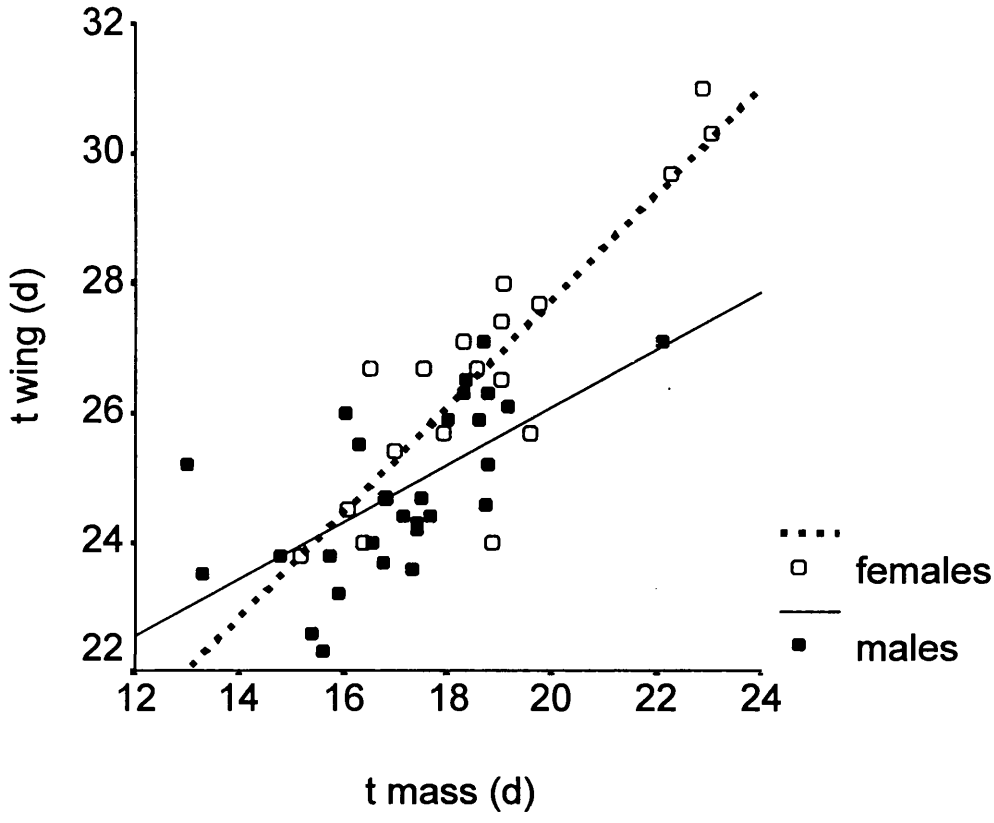

Figure 6.4: Time of maximum wing growth ($t_{i\text{-wing}}$) in relation to time of maximum mass gain ($t_{i\text{-mass}}$) for male and female great skua chicks. Only fledged chicks are included. Significances of predictor variables as determined by an ANCOVA with dependent variable $t_{i\text{-wing}}$ are: sex $F_{1,47} = 4.451$, $p = 0.041$; $t_{i\text{-mass}}$ $F_{1,47} = 67.639$, $p \leq 0.001$; sex*$t_{i\text{-mass}}$ $F_{1,47} = 5.962$, $p = 0.019$. Coefficient of $t_{i\text{-mass}}$ for females: 0.817 ± 0.111 (s.e.), $p \leq 0.001$; males: 0.443 ± 0.106 (s.e.), $p \leq 0.001$.

Sex-specific effects of hatching condition on growth (control and experimental chicks)

When including chicks from all treatment groups (control, foster and removal), condition at hatching was lowest for chicks emerging from sixth and seventh eggs (BMI control: 1.69 ± 0.81 (s.e.), $n = 47$; 4th eggs: -1.32 ± 1.21 (s.e.), $n = 24$; 6th and 7th eggs: -2.30 ± 0.50 (s.e.), $n = 28$; ANOVA: $F_{2,98} = 6.349$, $p = 0.003$). Hatching condition did not

have differential effects on growth for chicks in the three treatment groups (see appendix 6-B). Also, there was no significant interaction effect of treatment and sex for any of the growth parameters, i.e. the treatment affected growth of males and females in the same way (appendix 6-B). We therefore pool chicks from all treatment groups when analysing the effects of hatching condition on growth. Hatching condition had a sex-specific effect on the timing of mass gain. Females hatching in better condition reached maximum mass gain ($t_{i\text{-mass}}$) significantly earlier during their nestling period than those in poor condition (fig. 6.5). By contrast, hatching condition did not affect the time it took male chicks to reach $t_{i\text{-mass}}$. Linear growth rates of mass as well as wing and tarsus growth were not affected by hatching condition.

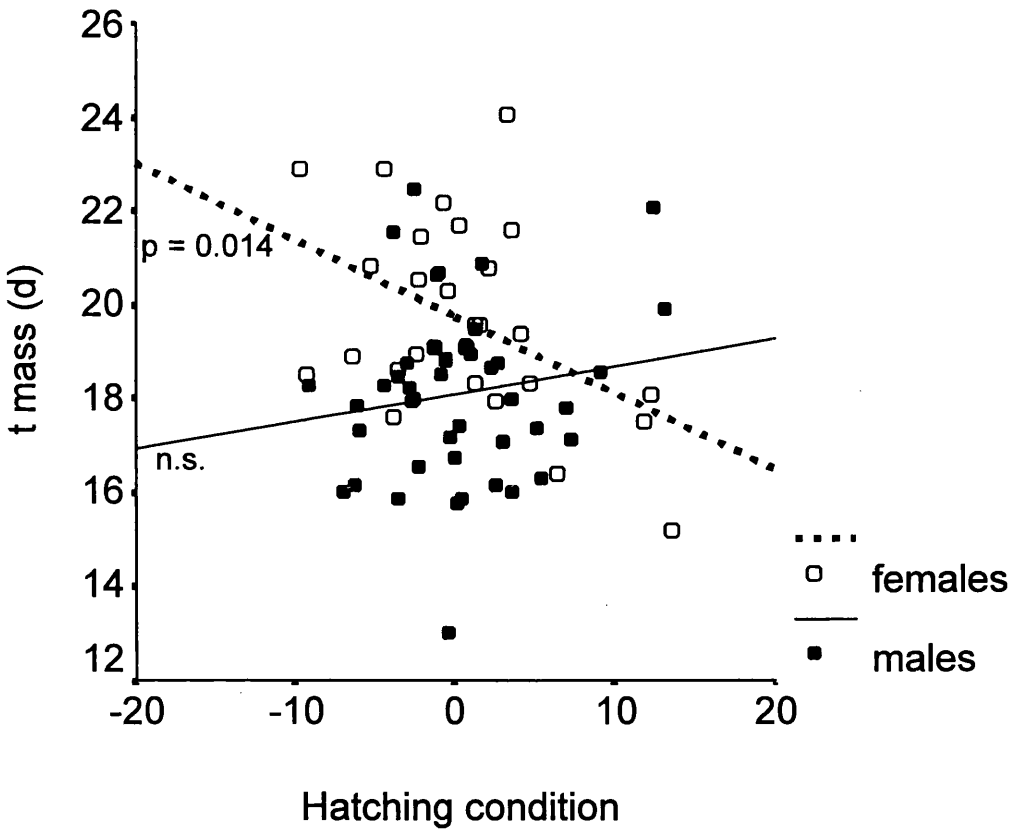

Figure 6.5: Time of maximum mass gain ($t_{i\,-\,mass}$) in relation to hatching condition (calculated as body mass index, BMI) for male and female great skua chicks. Significances of predictor variables as determined by an ANCOVA with dependent variable $t_{i\,-\,mass}$ are: sex $F_{1,72} = 13.527$, $p \leq 0.001$; BMI $F_{1,72} = 1.473$, $p = 0.229$; sex*BMI $F_{1,72} = 6.652$, $p = 0.012$. Coefficient of BMI for females: -0.163 ± 0.062 (s.e.), $p = 0.014$; males: $p = 0.329$.

Growth and survival (control and experimental chicks)

Some aspects of growth were strongly correlated to the survival probability of a chick. Even though the direction of causality is not clear, it is interesting to note that slow wing growth and a low linear growth rate were connected to poor survival (tab. 6.2). This connection was the same for both sexes of offspring. Growth of tarsus, by contrast, was not related to survival probability.

Table 6.2: Results of logistic regression examining the effects of growth parameters on survival probability of great skua chicks. Only the factors remaining in the parsimonious model are presented. No interactions of sex and growth were significant. In addition to the growth parameters, hatching condition and date were included in the full model. Only chicks measured at least to an age of 16 days are included in the model (n = 103). Significance of factors was determined by the change in total deviance if the factor is removed from the model.

	average effect	**s.e.**	**Wald statistic (χ^2)**	**d.f.**	**p**
$t_{i\text{-wing}}$	-0.265	0.112	5.617	1	0.006
Linear growth rate	0.092	0.047	3.830	1	0.041

6.5 Discussion:

The female-biased sexual size dimorphism that is found in adult great skuas (Catry *et al.* 1999, this study) is reflected in the development of the chicks. Even though hatching at the same weight and size, daughters reached a higher fledging mass and grew longer wings than sons. Interestingly, tarsus growth was not different between the sexes in any respect and tarsus length differs little between male and female adult great skuas (Furness 1987, this study). The extent of size dimorphism exhibited by nestlings near the time of fledging was almost identical to that seen in adults. Our results further show that sons and daughters reach fledging size of body mass and tarsus length within the same time span after hatching. Contrary to many other studies, our results do therefore not suggest that the smaller sex grows at a higher relative rate than the larger one (Ricklefs 1979; Richter 1983; Teather & Weatherhead 1994).

Ricklefs (1979) proposed that all observed growth rates (with the possible exception of pelagic seabirds) are maximised to physiological limits given the final size and ecology of the species. However, it appears that in order to achieve a larger final size during the same time period, female great skuas are able to invest relatively more into

growth than males. Some evidence for this comes from the fact that females exhibited a higher rate of daily mass increase than sons during the second half of the nestling period. On the other hand, this higher investment into body mass increment appears to be set off by a decreased ability to invest in feather growth. A negative relationship between mass and feather increment has also been found for the larger sex in a dimorphic passerine species (Richter 1983). Female great skua chicks reach maximum mass increase at the same age as male chicks, but only achieve maximum wing growth two days later in the nestling period than males. This female-specific trade-off between mass increase and wing growth becomes stronger amongst poorly growing chicks. The longer it took a female nestling to reach maximum mass increase, the longer the interval until she reached maximum wing growth in comparison to a male chick. These results provide strong evidence for sex-specific constraints of energy allocation during growth. Interestingly, only feather but not skeletal development seems to be affected by the increased investment into body mass gain by females. Feather growth is a highly demanding process (Murphy 1996), and it is crucial in order to fledge successfully. Several studies, including an earlier study on growth of great skua chicks, have found that during food restriction, feather increment is least affected (Furness 1983; Boag 1987; Smith & Wettermark 1989). This does not necessarily mean that feather development is less costly than mass or skeletal growth. On the contrary, it could imply a tight regulation on feather growth, as it can play a crucial role for immediate offspring survival (De Kogel 1997). Delayed feather development might increase the number of days spent on the territory until fledging, and thereby increase predation risk. It could also result in poor flight performance after fledging, which would make the fledglings more vulnerable to predators or intra-specific aggression and less efficient in foraging. These considerations suggest that there is a genuine trade-off between mass and feather growth in female great skua chicks.

Our results indicate that early development plays a crucial role for the performance of chicks, in particular for the larger sex. Hatching condition, which is tightly connected to the resources obtained from the egg (Williams 1994), affected mass gain of females but not males. Hatching in good condition allowed females to reach maximum mass gain earlier than those that hatched very light. Once again this suggests that females face higher developmental constraints in order to reach their final body mass than do males. Tighter constraints on the growth of the heavier sex have also been found in some other species, for example in capercaille (*Tetrao urogallus*) chicks (Lindén 1981). It is intriguing to note that despite the obvious mass difference between male and female great skua chicks not appearing until 15 to 20 days post-hatching, condition at hatching can determine later

performance of the larger sex. Chicks which reach a high postnatal growth rate tend to have a higher metabolic rate already at hatching (Klaassen & Drent 1991). In order to sustain a high level of tissue synthesis later, it is likely that they need larger support organs, such as liver or kidneys (Klaassen & Drent 1991). Additional to energy limitation, tissue specific constraints during early development are likely to be limiting for later growth performance. For example, it has been shown that young birds on a low protein diet show impaired development of some vital organs, such as the bursa of Fabricius and the spleen, thereby negatively affecting immunocompetence (Lochmiller *et al.* 1993). In rats it is known that maternal diet affects aspects of heart development in offspring (Morris *et al.* 1995), while in sheep, maternal nutrition during the first half of gestation influences tissue-specific gene expression after birth (Whorwood *et al.* 2001).

Apart from the direct connection between slow growth and a higher rate of mortality, highlighted by this study, slow growth is also likely to affect the post-fledging success of an individual (De Kogel 1997; Ohlsson *et al.* 2002). With respect to predictions made by sex ratio theory in relation to cost of differently sexed offspring, it is important to include the likely consequences of poor growth beyond simple mortality. As argued by Richner (1991), the cost of an individual offspring should be calculated in terms of the inclusive effect the raising of that offspring has on the residual reproductive value of the parents. Poor development during ontogeny has been connected with higher mortality and disease susceptibility as well as low reproductive success later in life (de Kogel 1997). Low reproductive potential of offspring in turn has a negative effect on the parents' reproductive value. As the larger sex is more affected by poor growth, it is likely to incur higher costs in that respect than the smaller sex. This would result in an increase in the cost of the large sex beyond the simple energetic cost of raising it. The issue of why this does not result in more skewed offspring sex ratios following Fisher's (1930) predictions remains unsolved.

We have shown that conditions during early development, in particular resources obtained from the egg, have a strong influence on the growth of female chicks, the larger sex, but much less so on males. We have also demonstrated that the larger sex faces a stronger trade-off between the development of different body parts, and slow growth in turn is related to a high mortality rate. However, an intriguing question remains: what is the physiological mechanism that renders females, or more generally the larger sex, more vulnerable to a shortage of resources, even during a time in development when there is no obvious size difference? In relation to large male offspring one of the main suspects has long been a high level of androgens circulating during development of sexual organs.

However, this explanation does not hold when females are larger, as it is very unlikely that reversed sizes are accompanied by reversed levels of androgens. Equally, heterogameity does not appear to present a strong sole explanation. In the closely related lesser black-backed gull and great skua offspring vulnerability is always found in the larger sex, despite it being the homogametic sex (males) in gulls and the heterogametic sex (females) in skuas (Griffiths 1992, Nager et al. 1999, Kalmbach et al. *in prep*). Even though both the above mechanisms are likely to have some effects (e.g. de Kogel 1997), we suggest that size-specific vulnerability is related to tissue specific nutrient demands in early life: such tissues or organs as the heart or the liver, which are needed in order to support fast growth later during ontogeny (Klaassen & Drent 1991).

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6.7 Appendix

A: Biometric measurements of male and female adult great skuas from Foula, Shetland, caught between 1999 and 2001. Result of t-test comparing male and female measurements. Length of sternum was measured with two fingers on the mid-point of the two clavicles, and is therefore referred to as sternum index. Asterixes indicate significant size differences between the sexes after sequential Bonferroni correction of the t-test results.

	males	**females**	**t**	**p**	
Mass (g)	1298.8 ± 88.6 (26)	1436.8 ± 77.4 (80)	7.615	0.000	*
Tarsus (mm)	70.4 ± 2.3 (16)	70.8 ± 2.2 (94)	0.614	0.541	
Wing (mm)	419.6 ± 8.7 (37)	428.0 ± 7.9 (123)	5.498	0.000	*
Sternum - index (mm)	90.8 ± 4.6 (25)	94.8 ± 3.6 (122)	4.777	0.000	*
Head-bill (mm)	111.3 ± 2.6 (25)	112.4 ± 2.6 (120)	1.798	0.074	

B: Results of ANCOVA testing for differential effect of hatching condition on growth in different treatment groups (condition*treatment interaction), and for treatment effect on sex-specific growth (treatment*sex interaction). The dependent variable was always the respective growth parameter, and predictor variables hatching condition, treatment, sex, condition*treatment and treatment*sex. Only the significances for the interaction terms are given. N = 72; 35 control, 19 foster and 18 removal chicks.

Dependent variable	**Condition*treatment**	**Treatment*sex**
k_{mass}	0.445	0.972
$t_{i\text{-}mass}$	0.276	0.986
k_{tarsus}	0.236	0.519
$t_{i\text{-}tarsus}$	0.257	0.866
k_{wing}	0.700	0.820
$t_{i\text{-}wing}$	0.397	0.898
linear growth rate	0.171	0.147

Chapter 7

Daughters are more expensive than sons in the great skua *Catharacta skùa*

Manuscript:

E. Kalmbach, R. W. Furness and S. Waldron. Daughters are more expensive than sons in the great skua, *Catharacta skua*

7.1 Abstract

In sexually size-dimorphic species, the larger sex is thought to require more energy until reaching independence. This assumption is an integral part of theories aiming to understand parental investment. However, there is a lack of direct measurements of daily energy expenditure (DEE) of male and female offspring. Doubly labeled water (DLW) is the most widespread method to measure energy expenditure in free-living animals. We used DLW to measure sex-specific differences in energy expenditure in free-living great skua chicks *Catharacta skua*, in which females are larger. Additionally we determined organ sizes of male and female chicks of different ages, as these can influence metabolic rates. DEE for offspring of both sexes scaled to body mass with a factor of 0.673. Male and female chicks did not differ in mass-specific energy expenditure, which ranged between 0.78 and 1.96 kJ/g/d. Due to their larger final size, females needed a higher amount of total metabolisable energy (TME) with 45152 kJ from hatching to fledging compared to males with 42388 kJ. The female:male ratio of TME is smaller than the size ratio at the end of the nestling period, thereby making the larger sex cheaper to raise per gram despite the higher absolute amount of energy needed. Values of energy expenditure obtained for great skua chicks lie within ranges determined across a wide range of species. Organ sizes did not differ between sexes at any age, but further investigation of body composition is needed. Sex ratio at fledging in one year was more male biased than predicted by TME ratio, while hatching sex ratio over two years reflected offspring cost.

7.2 Introduction

In animal species which exhibit sexual size dimorphism, it is generally thought that the larger sex is more costly to produce for parents than the smaller one (Clutton-Brock *et al.* 1981; Anderson *et al.* 1993; Ono & Boness 1996; Daunt *et al.* 2001). The existence of differences in production costs between the sexes lie at the heart of theories aiming to predict parental investment into the two sexes (Fisher 1930; Trivers & Willard 1973; Charnov 1982; Clutton-Brock 1991). However, it has been difficult to obtain widespread empirical data to support these theoretical predictions, particularly for birds, which are otherwise widely used as model organisms for life history studies (Gowaty 1991; Emlen 1997). For example, Fisher's (1930) sex ratio theory predicts that the populational offspring sex ratio should reflect the differential rearing costs and therefore be skewed in favour of the smaller sex. In some extremely sexually size-dimorphic species, this prediction does not hold true when empirical data are collected (Fiala & Congdon 1983; Richter 1983; Anderson *et al.* 1993). The lack of clear support for theoretical predictions has in part been attributed to mechanisms which could render the smaller sex more costly or the larger sex less costly than expected simply from their size. For example, the larger sex might have evolved ways to use energy more efficiently during growth, resulting in a lower than expected energy expenditure (Teather & Weatherhead 1994). Alternatively, the smaller sex might need to be more mobile in order to compete successfully with larger siblings, which would result in a higher energy expenditure than expected (Torres & Drummond 1999). Although a higher energetic cost of the larger sex is supported by several studies of bird species where males are larger (Lindén 1981; Fiala & Congdon 1983; Teather & Weatherhead 1988), in species where females are larger, evidence is still scarce and ambiguous (Newton 1978; Riedstra *et al.* 1998; Torres & Drummond 1999). Some male-specific costs have been proposed, regardless of size. Higher levels of androgens during development for example, might increase male metabolic rate, whether they are larger or not (Wikelski *et al.* 1999). Differing costs connected to aspects other than size could reflect differences in body composition. For example, a higher mobility in one sex should result in a larger muscle mass, particularly in the leg muscles in species where chicks are highly mobile from a very young age. On the other hand, faster growth rates and a higher metabolic rate might be reflected in organ sizes, particularly organs which are involved in tissue synthesis (Daan *et al.* 1990; Klaassen & Drent 1991).

While many studies have reported values of adult energy expenditure, only very few have directly measured field metabolic rates of chicks (e.g. Fiala & Congdon 1983; Klaassen & Drent 1991; Gabrielsen *et al.* 1992; Riedstra *et al.* 1998). This is mainly due to

the previously widely held opinion that the doubly labeled water method, which is commonly used to measure field metabolic rate in animals, might produce large errors when measuring energy expenditure in growing chicks (Visser & Schekkerman 1999). However, recent validation studies give strong support for the applicability of this method in chicks, if corrections are made accounting for a different rate of evaporative water loss (Klaassen *et al.* 1989; Visser & Schekkerman 1999; Visser *et al.* 2000). We used the doubly labeled water method to measure energy expenditure in great skua, *Catharacta skua*, chicks. In great skuas, adult females are heavier and slightly larger than males, and this size difference between the sexes starts to develop in the first half of the nestling period (chapter 6). Also, female chicks exhibit a higher mortality than male chicks (Kalmbach *et al. in prep*), and when in poor condition, mothers produce more sons than daughters (Kalmbach *et al.* 2001). This indicates that female chicks of the great skua are more costly to produce than male chicks. As metabolic rate varies with relative organ sizes (Daan *et al.* 1990), we also examined whether there are sex-related differences in body composition in great skua chicks. For this we used freshly dead chicks found at random in the field. Relying on chicks found dead is prone to producing a biased sample, as these chicks might have died of starvation. However, in great skuas a high rate of chick mortality is due to intra-specific aggression (Hamer & Furness 1993), which allowed us to obtain fresh carcasses from chicks which had not apparently died from starvation. We predicted that if one sex incurs costs which are not proportional to its size, we were likely to see this in a relative change of body composition. However, if it is only size which determines the energetic cost of offspring, body composition should not vary greatly between the sexes, and energy expenditure should mirror the size difference.

7.3 Methods

Daily Energy Expenditure (DEE) of great skua chicks was measured using doubly labeled water during the first two weeks of July 2000 on Foula, Shetland (60°08'N,2°05'W). Carcasses of dead chicks were collected between June and August 2000 on Foula. All chicks were sexed molecularly, following the method described by Griffiths *et al.* (1998) with the alterations given by Kalmbach *et al.* (2001).

Doubly-labeled water / energy expenditure

Fifteen chicks each were used for the doubly labeled water analysis on the 5 July and 10 July 2000. Chicks were ringed, weighed and wing length was taken, before approx. 1ml of doubly labeled water mixture was injected intraperitoneally. The chicks ranged in

body mass at the time of injection from 260 to 1120 g. The doubly labeled water mixture was obtained by mixing 10 ml of 95 atom % $H_2{}^{18}O$ labeled water with 25 ml of 99.9 atom % of ^2H_2O labeled water. This resulted in 35 ml of water with 27.14 atom % ^{18}O and 71.36 atom % ^2H, to which we added a mixture of salts to make up isotonic chicken saline. The quantities of salts we added to the 35 ml of water were: NaCl: 0.301 g, KCl: 0.0108 g, $CaCl_2$: 0.007 g, KH_2PO_4: 0.007 g, $MgCl_2$: 0.0035 g and glucose: 0.07 g. Two hours after the injection an initial blood sample was taken from the tarsal vein. and the chick was released on its territory where it had been found. The blood samples were flame-sealed in capillaries, following the procedure described by Tatner and Bryant (1989). A further blood sample was taken 24 and 48 hours after the initial sample had been obtained and in each instance the chick was weighed. Not all chicks were found at each sampling time. Samples from chicks that were recaptured were mostly obtained within a few minutes of the exact 24 hour period (average time span 23 hrs 59 min ± 9.6 min, n = 61), the largest deviation from the exact 24 hour time span was 30 minutes. When calculating the average energy used per hour we corrected for these variations by using the exact time span between sampling points for each chick. Additionally we took blood samples from six other chicks to be used as background samples for the isotopic signature of body water from great skua chicks on Foula.

The isotopic composition of the blood samples was analysed at the SUERC stable isotope facility in East Kilbride, UK. Measurement of the ^{18}O / ^{16}O ratio was carried out using the guanidine method described by Tatner and Bryant (1989). For the ^2H / H analysis water was extracted from blood using refrigerated ultra filtration. Contents of up to three capillaries per sampling point of each bird were pooled for centrifugation, and samples were spun at 10 °C and 10k rpm for 90 minutes. Samples were weighed before and after centrifugation with an ultra fine balance with an accuracy of ± 0.00001g. Immediately after centrifugation the clear serum was taken up and flame-sealed into 50 µl vitrex capillaries. Prior to the analysis we carried out a calibration experiment of the filtration process using water standards of known isotopic composition. A small but consistent isotopic fractionation occurred during filtration, which depended on original sample volume and enrichment. We thus corrected all the values obtained from our samples accordingly. The hydrogen isotopic analysis of the serum samples was carried out by reduction to hydrogen using the chromium furnace reduction method described by Donnelly *et al.* (2001).

For the conversion of isotopic abundance (ppm) into daily energy expenditure (DEE) by the chicks we followed the calculations of Tatner and Bryant (1989, p 78). To

estimate the body water pool we used data obtained from the carcass analysis of six chicks, between 94 and 1060 g (average body mass 400.3 ± 358.9 g). The average body water content was 73.2 ± 6.5 % of body mass. As value for body mass of each chick and each sampling period, we used the average mass between sampling points. To calculate the CO_2 production from the turnover rates of the isotopes, we followed the basic calculation by Tatner and Bryant (1989), but with corrections for the evaporative water loss of growing chicks as suggested by Visser and Schekkerman (1999). Visser and Schekkerman suggest using an estimated evaporative water loss of 25 %, when the real value is not known. We decided to use the value of 20%, which had been determined for Arctic tern chicks (*Sterna paradisaea*; from Visser & Schekkerman 1999 who re-analysed data from Klaassen *et al.* 1989), as terns are reasonably closely related to skuas, and tern chicks exhibit semi-precocial development, as do skua chicks. As conversion factor from CO_2 production to kJ we applied the commonly used value of 1.0 l CO_2 = 27.3kJ (Gessaman & Nagy 1988).

We aimed to obtain isotopic measurements for two consecutive 24 hour periods from each chick. However, not all chicks could be recaptured twice and additionally some samples were lost due to broken capillary seals. For most chicks we obtained at least one value of energy expenditure over a 24 hour period. However, variation of energy expenditure was greatly reduced when only those chicks were included for which samples of the full 48 hours were available. In the analysis we therefore only included chicks for which data of both 24 hour periods were available, and averaged energy expenditure over the whole time-span of 48 hours. Including all chicks for which only one 24 hour measurement was available did not change the overall conclusions, only the exact value of the exponent in the equation relating body mass to energy consumption (see section 7.4).

From the measure of DEE, which only includes metabolic heat production, we calculated the daily metabolisable energy (DME) used by each chick, which also accounts for the energy fixed in new tissue by growing chicks:

$$DME = DEE + (ED_f - ED_i) \quad kJ/d$$

ED_i and ED_f represent the energy density of the body at the beginning and end of a measurement. For the energy density of chicks with a given mass M growing towards the asymptotic weight A we used average values given by Weathers (1996, p. 473):

$$ED = M\,(3.51 + 4.82 * M/A),$$

as we did not analyse the dried carcasses we had obtained for their calorific value. We repeated these calculations with values of energy density reported for kittiwake (*Rissa tridactyla*) chicks (Gabrielsen *et al.* 1992), which essentially produced the same results. By

employing the same value of ED for both sexes, we assume a non-distinct body composition of male and female chicks. This assumption is supported by our carcass analysis (see section 7.4). The total metabolisable energy (TME) represents the amount of energy a chicks needs from hatching until fledging.

Body composition

Bodies of dead chicks were frozen at –21 °C until dissection. After thawing, tarsus, wing and head-bill length were measured and fresh weight of the carcass was determined. Carcasses were dissected and the following organs were removed: left and right pectoral muscle, liver, heart, kidneys, stomach, intestines and right tarsal muscle. In very young chicks the yolk sack was still present, which in this case was also removed. The fresh weight of all organs was noted before they were dried at 65 °C for several days, until they had reached a stable mass, which was recorded as dry weight. Of all chicks a blood or tissue sample was taken for molecular sexing.

Statistical analysis

We tested for differences in mass-specific energy expenditure between the sexes by employing an ANCOVA design with the explanatory variables sex, mass and the interaction sex*mass. The parsimonious model was determined by backward elimination. In order to achieve linearity between mass and energy expenditure we log-transformed the data prior to the analysis. In order to scale energy expenditure to body mass we fitted allometric growth equations of the form $y = b*mass^a$ using the non-linear curve fitting procedure in SPSS for Windows, version10. All test were two-tailed and the level of significance set at 5 %.

7.4 Results

Daily Energy Expenditure (DEE) over a 48 hour period could be determined for 14 female and 8 male chicks, ranging in body mass from 260 to 1120 g at the beginning of the measurement period (average female mass: 692 ± 304 g; average male mass: 748 ± 174 g). DEE was related to body mass for both sexes through the equation

$$DEE = 10.61 * mass^{0.673} \quad kJ / d$$

Mass-specific energy expenditure (kJ / g / d) was not different for male and female chicks (ANCOVA with dependent kJ/g/d: mass p = 0.003; sex p = 0.461, mass*sex p = 0.660). Energy expenditure per gram decreased non-linearly with increasing body mass (fig. 7.1).

The relationship between body mass and mass-specific energy expenditure (kJ / g / d) for both sexes can be described by the equation

$$EE = 7.97*mass^{-0.281} \quad kJ / g / d$$

As female chicks are heavier from around 20 days of age, daily energy expenditure (DEE) of daughters exceeds that of sons in the second half of the nestling period (fig. 7.2a). DEE, as measured with doubly labeled water, only accounts for the metabolic heat production, or in other words the respiratory energy used by the bird. We calculated daily metabolisable energy (DME) for male and female chicks, which represents the amount of energy used for non-growth activities, such as motor activity, thermoregulation and digestion, plus the energy deposited in growing tissue. DME of both male and female chicks peaked at the beginning of the second half of the nestling period, but females' maximum DME was two days later than males' (fig. 7.2b; peak DME females at 28 days: 1452.1 kJ, peak DME males at 26 days: 1338.5 kJ). The daily energetic demands of daughters exceeded that of sons after an age of 16 days and remained higher until fledging. By the end of the nestling period (at 45 days), the difference in DME amounts to a difference in the total metabolisable energy (TME) a male (42388 kJ) or female (45152 kJ) chick consumes from hatching to fledging. The ratio of TME at fledging between female and male chicks was 1.07, while the ratio of body mass was 1.14 (see chapter 6).

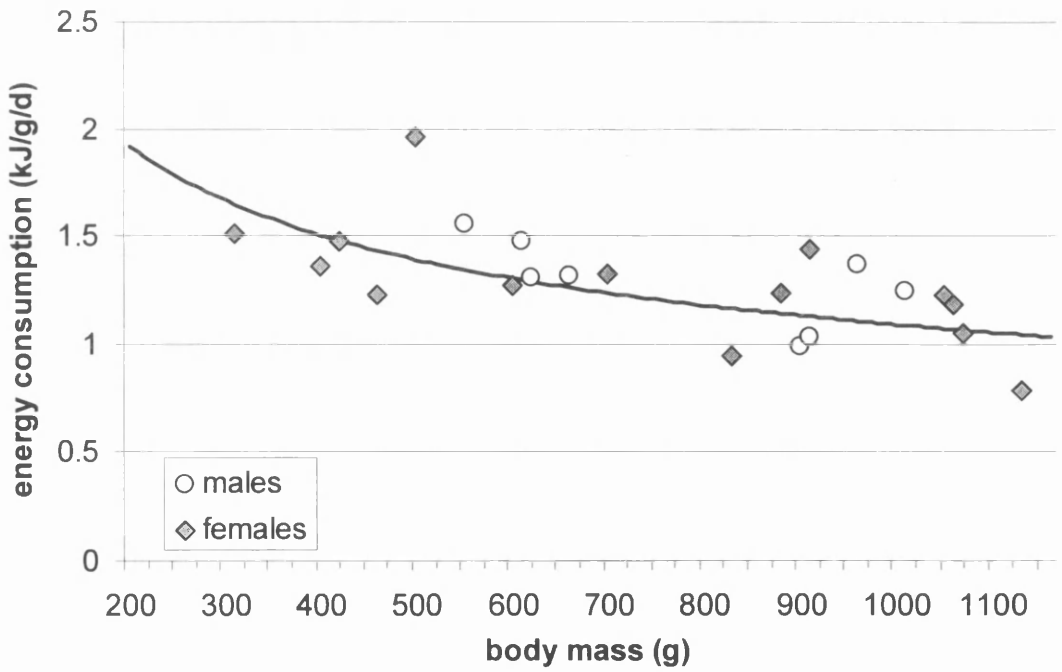


Figure 7.1: Mass-specific energy consumption of male and female great skua chicks. Only chicks included for which energy consumption was measured over 48 hours.

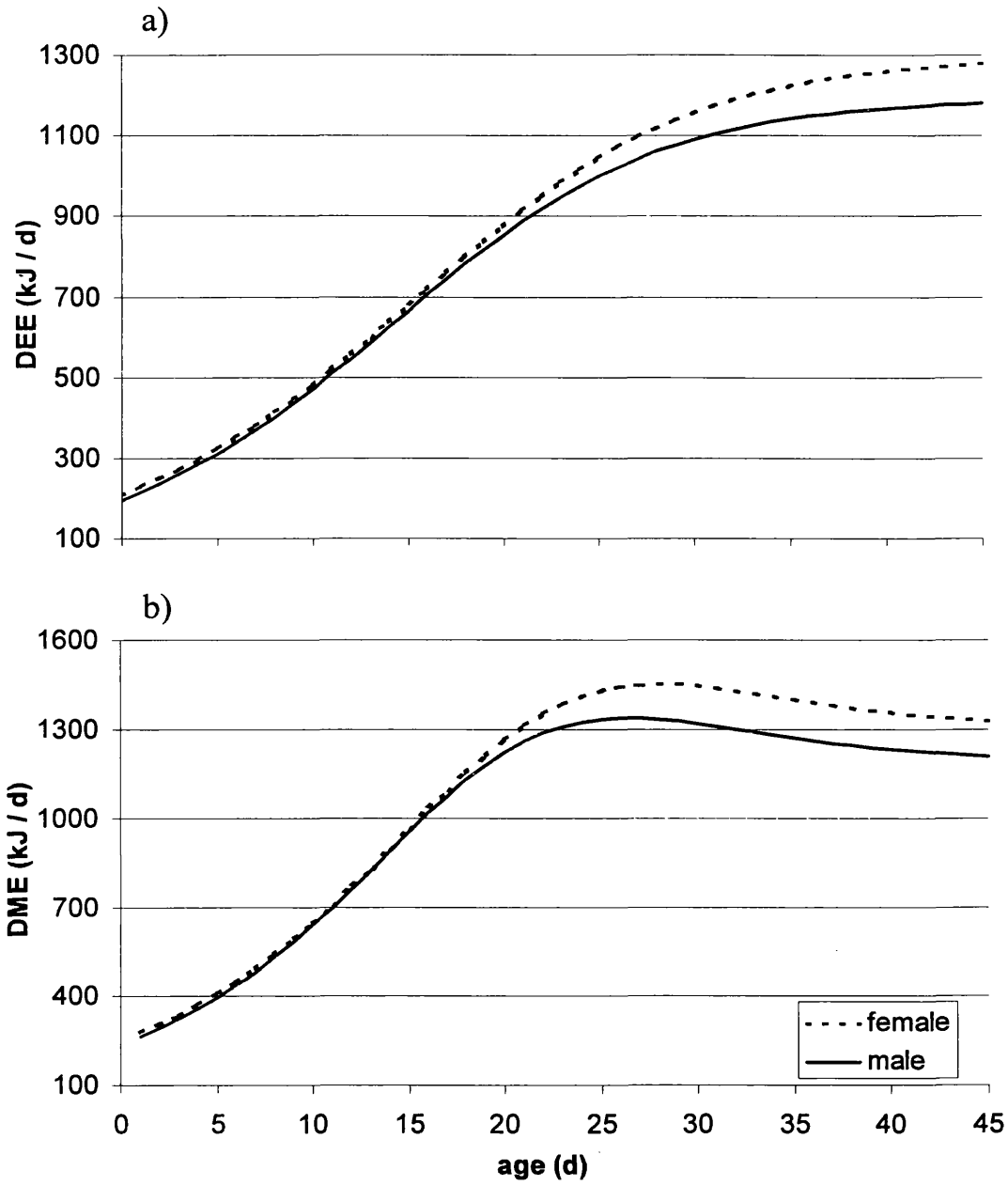
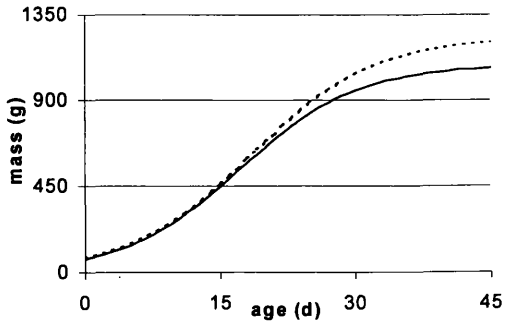


Figure 7.2a, b: Daily energy expenditure (DEE) and daily metabolizable energy (DME) of male and female great skua chicks. For comparison small figure shows growth of males and females, reproduced from chapter 6 (fig. 6.2a).



Body composition was determined for 11 male and 12 female chicks, ranging in size from chicks which had died at hatching with a fresh mass of 36.17 g to a maximum body mass of 1060 g. Three of those 23 chicks were obviously undernourished and therefore not included in the comparison of body organs between males and females. Of the remaining 20 chicks, the largest proportion was made up of hatchlings (average body mass: 61.7 ± 8.9 g; n = 12). Yolk made up the largest proportion of the newly hatched chicks, about ten times more in weight than the largest single other organ, the liver (fig. 7.3). There were no statistical differences in organ sizes between male and female hatchlings (fig. 7.3). With the exception of the pectoral muscle, the weight of internal organs increased linearly over the age of chicks (fig. 7.4). Bearing in mind our relatively small sample size, there were no indications of differences in organ sizes relative to age or body size (head-bill measurement) between male and female chicks (ANCOVA design with dependent variable = respective organ dry weight; model I: explanatory variable sex, age and interaction sex*age; model II: explanatory variable sex, head-bill length and interaction sex*head-bill; interaction terms and main factor sex in all tests n.s.; while age and head-bill were highly significant for all organs with the exception of pectoral muscle).

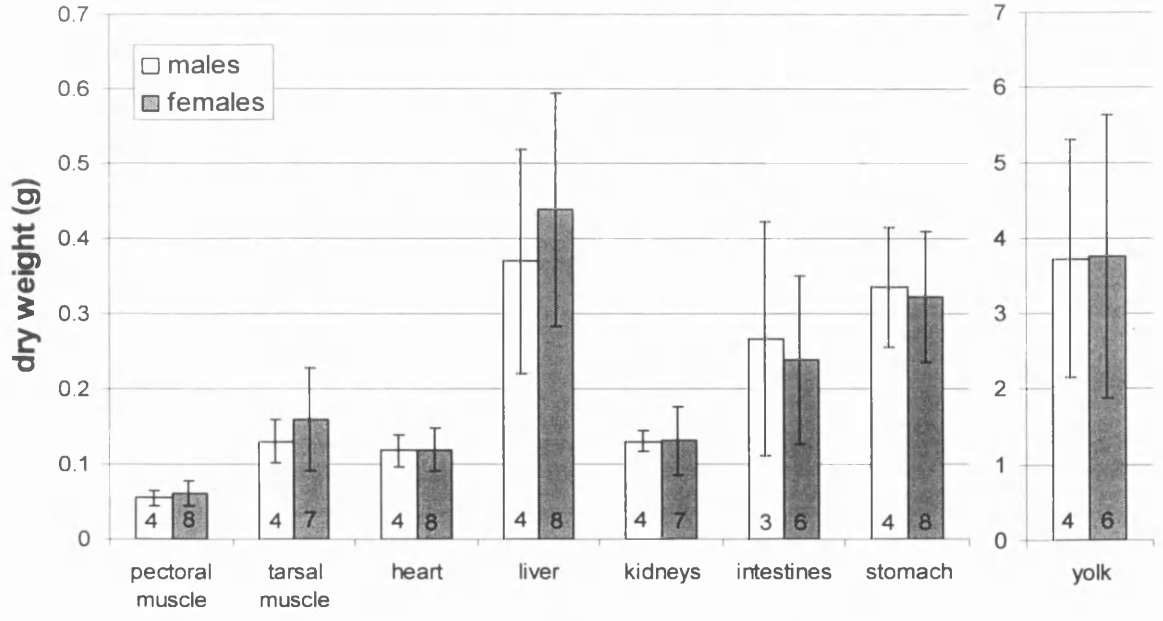


Figure 7.3: Dry weight of body organs of hatchling male and female great skua chicks. Note the scale for yolk is x 10 the scale of the other organs. None of the organs differed significantly in size between the sexes (t-tests, all n.s.). Numbers inside bars denote sample sizes.

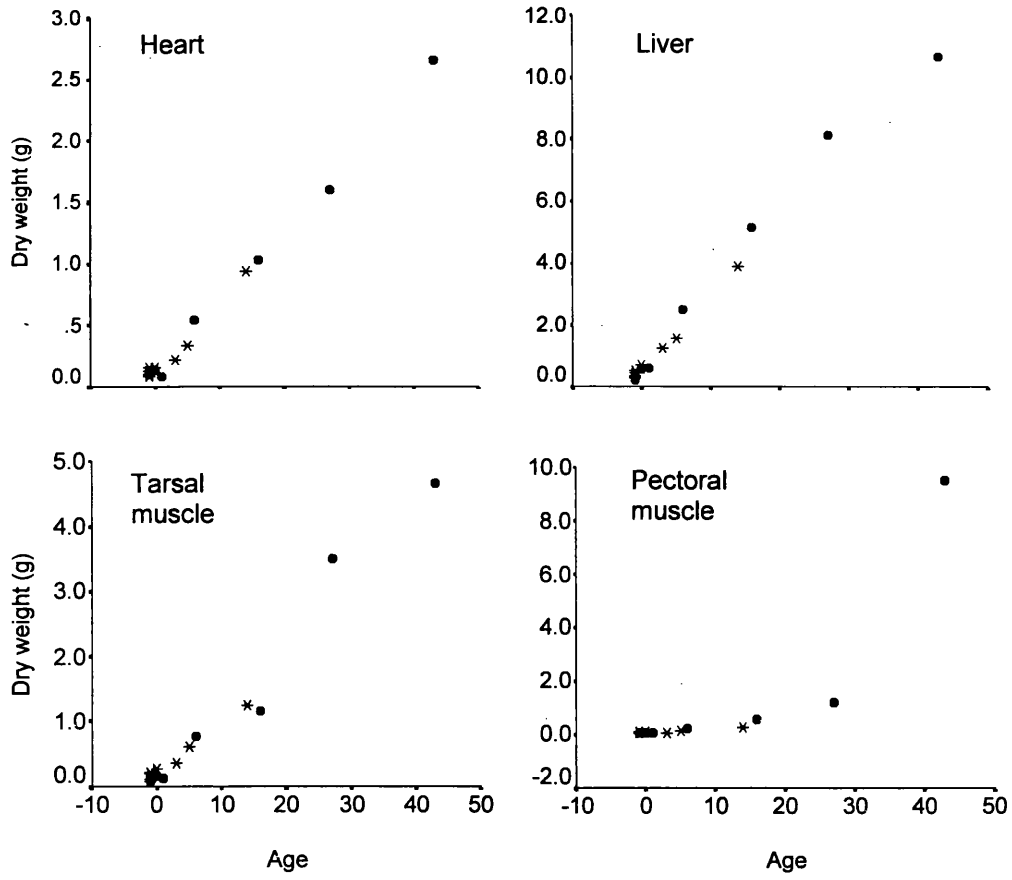


Figure 7.4: Dry weight of organs over chick age for male (•) and female (✳) chicks.

7.5 Discussion

In the great skua, female chicks are energetically more expensive to produce than male chicks. The difference in energy required until fledging (TME) reflects the size difference at the end of the nestling period, when daughters are larger than sons. However, the female:male ratio of energy required until fledging was smaller than the ratio of fledgling masses. A lower per gram cost for larger birds has been demonstrated intra- as well as interspecifically (Weathers 1996; Krijgsveld *et al.* 1998). Production costs for a given-sized fledgling are amazingly constant across the whole range of bird species (Weathers 1996). Most of the variation (97 – 99 %) in energy requirements until fledgling can be explained by the final mass and the length of the fledgling period. Great skua chicks are no exception from this, as the values we obtained match those predicted by equations derived from many different species to a high degree (all equations taken from Weathers 1996). TME was slightly higher for great skua nestlings (males: 11 %, females: 9 % higher) than predicted by final mass and fledging period alone. Equally, average daily metabolised energy (ADME) was slightly higher (15 and 12 %), while peak DME almost

exactly matched the predicted values. Therefore our results provide further evidence for the general pattern that in size-dimorphic species the larger sex is proportionally cheaper to raise per gram than the smaller sex (reviewed in Krijgsveld *et al.* 1998). Daily energy expenditure (DEE) scaled to body mass with a factor of 0.673. This represents a slightly steeper increase of energy expenditure with body mass than has been found for seabirds in general (reproducing seabirds: 0.611, non-reproducing seabirds: 0.530; Koteja 1991). The steeper slope for great skua chicks might reflect the increased activity of older chicks, such as wing flapping. A slightly higher energy requirement than predicted from the literature, from equations derived for many different species, is likely to reflect latitudinal effects. Birds in higher latitudes generally exhibit higher metabolic rates (Ellis 1984; Bryant & Furness 1995).

Energy expenditure per gram scaled with body mass with the factor –0.281 for chicks of both sexes. The inference that the difference in total energetic cost of male and female chicks is solely a consequence of the difference in final body mass between the sexes is underlined by the non-distinct mass-specific metabolic rates. Hence, we did not find any evidence that males incur higher energetic costs due to a male-specific physiological trait, as might be caused by high androgen levels during development (Folstad & Karter 1992; Wikelski *et al.* 1999). Equally, we did not detect any changes in body composition of the larger versus the smaller sex (Klaassen & Drent 1991). However, our sample size of dead chicks was relatively small, and therefore the question of sex-specific body composition warrants further investigation. Earlier results on sex-specific mortality in the great skua suggested that the amount of resources obtained from the egg, and the time directly after hatching, are particularly important for the successful development of females, but less so for males (Kalmbach *et al. in prep*; chapter 6). This difference in performance might be connected to differential resource allocation and higher metabolism of the larger sex (Klaassen & Drent 1991). As for chicks of all ages, we did not find differences in body composition of males and females at hatching. However, our sample of hatchlings included only four males and eight females, and in order to have a 90 % power of detecting a difference of the magnitude, seen for example in the tarsal muscle, a sample of 40 chicks of each sex would be necessary (for power analysis see Zar 1999, p 134). We were not able to measure DEE of hatchlings directly, but inferred their energy expenditure from the equation obtained for chicks between 260 and 1120g. It is possible that our interpolation is not exact for very small chicks, and we might therefore not have been able to detect small but crucial differences in energy expenditure between the sexes during the first few days after hatching. Determination of energy expenditure at

and just after hatching is particularly problematic with doubly labeled water, as the errors produced by this method are extremely large in very young chicks (Gabrielsen *et al.* 1992).

Fisher (1930) postulated that the relative cost of rearing one sex of offspring should be reflected in the sex ratio at independence. In some species a close match between cost and sex ratio has been found (e.g. rook *Corvus frugilegus* (Slagsvold *et al.* 1986), marsh harrier *Circus aeruginosus* (Riedstra *et al.* 1998)), even though it has been argued that the skewed sex ratios could be due to non-adaptive mortality patterns of the larger sex (Weatherhead & Teather 1991). In the great skua the ratio of energy consumed until fledging would predict an offspring sex ratio of 52 % males and 48 % females. In 2000, we found a fledging ratio of 60 % males and 40 % females in 51 broods, while the hatching ratio was 52 % males and 48 % females (n = 84 eggs). Over a period of two years we found a populational hatching ratio of 52 % males and 48 % females (n = 279 eggs), but this was not significantly different from a 0.5 distribution. It appears that in the great skua the hatching sex ratio might mirror the energetic costs of the differently sexed offspring, while the skew towards males at fledging is a non-adaptive consequence of high female mortality. This is in agreement with previous results, where we have shown that great skua mothers have a capacity to skew the primary sex ratio (Kalmbach *et al.* 2001), and female chicks show higher mortality (Kalmbach *et al. in prep*). However, more data on great skua fledging sex ratios are needed in order to test Fisher's (1930) predictions on a larger scale.

7.6 References

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Chapter 8

Inter-seasonal reproductive costs of increased egg production

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8.1 Abstract

Life history theory assumes that reproduction is costly. Reproduction can incur immediate or delayed costs to the parents in the form of lowered adult survival or reproductive output. Studies on the costs of reproduction have long focussed on the costs of chick rearing, but much less is known about the inter-seasonal costs of egg production. In the present study I experimentally increased egg production effort in female great skuas, *Catharacta skua*, in two years and followed their fate and breeding status in up to two breeding seasons after the manipulation. In the post-experimental year, return rates were not affected by the increased egg production, but females laid significantly later than in the previous year and later than control birds in the same year. Breeding success declines with laying date. Two years after the experimental increase in egg production effort, laying dates had advanced again to pre-manipulation values. Pair bonds and egg sizes were not affected. Within the experimental group, females which had raised chicks had a lower survival rate and a higher incidence of break-ups of pair bonds than those that had no chicks. These results indicate that in the long-lived great skuas increased egg production incurs inter-seasonal costs in the form of reduced reproductive output, while chick rearing incurs survival costs.

8.2 Introduction

The existence of a trade-off between current and future reproduction is a central prediction of life history theory (Roff 1992; Stearns 1992). This cost of reproduction can be paid in two different currencies, as increased adult mortality or as lowered reproductive output (Lessells 1991; Golet *et al.* 1998). Species with different life histories are expected to show a varying degree of balance between these currencies. For long-lived animals, a single breeding event is less important with respect to lifetime reproductive output than for short-lived animals. Equally, adult survival is of different importance for an animal which can reasonably expect to reproduce only during very few seasons or one that might breed for many years (Lessells 1991). As well as these costs of reproduction that are incurred after breeding (delayed costs), animals also incur immediate costs during breeding (Dawson *et al.* 2000). It is generally assumed that the two are connected, and that an increase of immediate costs also leads to an increase of delayed costs of reproduction (Oppliger *et al.* 1996; Dawson *et al.* 2000). As it is easier to measure direct costs of reproduction, many more studies have done that than measuring inter-seasonal costs of reproduction (Heaney & Monaghan 1996; Monaghan *et al.* 1998; Moreno *et al.* 1999; Veasey *et al.* 2001).

Birds have been used as model species by many biologists to test the predictions about reproductive costs in relation to different life histories. The clutch sizes we observe in nature are thought to reflect the balance of costs and benefits of each reproductive event. Lack's (1947) hypothesis that the number of eggs parents produce is ultimately restricted by the number of chicks they can raise successfully has been very influential on the study of the costs of reproduction. As the chick-rearing stage was deemed to be by far the most costly aspect of reproduction, experimental manipulations of reproductive costs have long focussed on this period. Brood enlargement experiments have been the most common way to make reproduction more costly and study the consequences of the increased cost on immediate and future reproductive success and adult survival (Gustafsson & Sutherland 1988; Hochachka 1992; VanderWerf 1996). However, in many studies the negative effect of raising an increased number of chicks on components of fitness was less than expected from theory (Stearns 1992; Orell *et al.* 1996; Murphy *et al.* 2000). Raising additional chicks alone did not seem costly enough to restrict clutch sizes to those most commonly found in nature (Monaghan & Nager 1997). Only recently have researchers started to investigate the reproductive costs involved in the production and incubation of the eggs in more detail (Monaghan *et al.* 1995; Oppliger *et al.* 1996; Cichon 2000). So far, only two experimental studies have been published that report inter-year reproductive costs of

increased egg production (Nager *et al.* 2001; Visser & Lessells 2001). Both studies indicate that the inter-year costs involved in egg production and incubation are substantial, even in comparison to chick rearing. Unlike the chick-rearing costs, most of the costs of egg production, and to a varying degree also incubation, are expected to be borne by the female.

We have previously shown that increased egg production effort in the great skua *Catharacta skua* incurs immediate costs for females in terms of decreased body condition (chapter 3). In the present study I investigate the indirect costs of experimentally increased egg production effort in females of this long-lived seabird in terms of survival and future reproduction. I followed the fate of individually marked females, which had been part of one of two different egg removal experiments, for up to two years after the manipulation. In 1999, we conducted an experiment in which the females laid on average three times more eggs than normal (two eggs), but did not incubate any eggs (Kalmbach *et al.* 2001). In the following year, we carried out a similar experiment with another group of females, but this time the birds also incubated eggs and, if they hatched, raised chicks (Kalmbach *et al. in prep*). Great skuas start reproducing at an age of four or five years, and can live up to 30 years (Furness 1987). Adult great skuas have a survival rate of around 80 to 95 %, are highly philopatric and form strong pair bonds, which can last for many years (Catry *et al.* 1997; Ratcliffe *et al.* 2002). Re-mating has been shown to carry a cost in terms of delayed breeding as well as reduced clutch volume and fledging success (Catry *et al.* 1997). If the immediate costs incurred during the season of increased effort are connected to inter-seasonal delayed costs, I expect to see some changes in survival or breeding behaviour in the year(s) following the experimental manipulation. Since the great skua is a long-lived, iteroparous bird, I predict that adult welfare is of higher importance than reproductive success in a given year, and that the currency of reproductive costs is lowered reproductive output rather than adult survival.

8.3 Methods

For the present study, I recorded return rates, pair bonds, laying dates and egg sizes of great skuas on Foula, Shetland (60°08'N,2°05'W), over a period of three years, from 1999 to 2001. Great skuas have been studied on Foula for more than thirty years, and many of the birds in the study population are marked with individual colour rings. In each of the study years, data were collected over several months, from prior to egg laying until at least hatching. Daily checks were carried out, during which we searched for colour ringed individuals, mapped breeding territories and recorded newly laid eggs, which were marked

and measured. Great skuas are highly philopatric and usually return to the same breeding territory year after year. It has been shown in previous studies that the re-sighting probability for a bird that is present in the colony is very high (Ratcliffe *et al.* 2002). Even when changing partner, great skuas tend to stay very close to their old territories (Catry *et al.* 1997).

The birds included in the analysis belong to three different treatment groups – two experimental groups (from different years) plus un-manipulated control birds. Additional to using previously colour ringed females as control birds, new individuals were marked each year. Efforts for recording laying dates and egg sizes of control birds varied between the years, which leads to differing sample sizes in different years and for different parameters. Overall, 131 females formed the control group, some of which might only contribute to return rates while others contribute to all parameters. Since the control group is a reasonably large group, we are confident that the repeated inclusion of some females over the three years does not bias our results. For laying dates, for example, only 17 females contribute to each of the three years.

The experimental groups are formed by females, which were part of an egg removal experiment in either 1999 or 2000. Experimental females from 1999 will be referred to as Exp1999 and those from 2000 correspondingly as Exp2000. Both experiments have been described in detail elsewhere (for 1999 see Kalmbach *et al.* 2001, for 2000 see Kalmbach *et al. in prep*). In both years, each new egg was removed from the nest on or the day after it was found, which induced females to produce several more eggs than the normal clutch of two. In 1999, egg removal was continued until the birds stopped re-laying, producing an average number of 6 eggs. Therefore, Exp1999 females faced increased egg production costs, but did not incubate eggs or care for chicks. In 2000, eggs were removed until the production of a sixth egg, which was then left in the nest for the parents to incubate. Of the 55 females where I started egg removal, 52 produced a sixth egg. Of these adults, 42 were individually colour-ringed. Within the experimental groups of each year there was one further subdivision. In 1999, half of the pairs received supplementary food for at least ten days prior to laying and during the egg laying stage. The Exp1999 group therefore consists of fed and un-fed females. In 2000, not all of the eggs left for the parents to incubate hatched chicks, which leads to a group of pairs which cared for a chick for a varying number of days (until the chick either died or fledged), and one that did not. For the present study, females are mostly grouped by their experimental year (Exp1999 n = 45, Exp2000 n = 42), regardless of the treatment group within the year. However, all data were also analysed according to the subdivisions of each experimental

group, but the results of these comparisons are only presented when there were differences between the sub-groups.

Not all females in the experimental groups were colour marked, either previously or during the experimental year. In several pairs only the male was colour ringed or, in a few cases, the birds were identified by plumage markings. In 2000, most females were un-marked prior to the experiment, but were caught and ringed during the course of it. In 1999, by contrast, only three of the previously un-ringed experimental females were marked. To analyse the impact of increased reproductive effort on the occurrence of partner changes in the year following manipulations, I restrict the sample to the Exp2000 group. As many of those females were previously unknown to us, they are likely to represent a random sample across different lengths of previously existing pair bonds. However, in 1999 most of the individually known birds of the experimental group had bred in the same pair bonds for several years previously, which might bias the rate of re-pairing. I only included pairs in which both partners were individually known in the analysis of pair bonds.

For the statistical analysis of binomial variables, such as return rate or divorce, I employed logistic regressions. Laying dates are given as medians and tested by non-parametric statistics. Statistical analysis was carried out using SPSS for Windows, version 10, and all tests are two-tailed.

8.4 Results

Return rates

Return rates of experimental females in the year following the manipulation were not different from control females (tab. 8.1). However, within the group of Exp2000, females which did not hatch any chicks had a higher return rate than those that hatched a chick (logistic regression, return rate dependent on 'with or without chick' $p = 0.046$, $n = 42$, tab. 8.1). Experimental females which had not cared for any chicks also returned at a higher rate than control females (logistic regression, return rate dependent on 'exp2000 without chick or control' $p = 0.041$, $n = 136$). In contrast to the females, male return rates did not differ between any treatment groups in either year (Control in 2000: 85 %, $n = 103$, in 2001: 80 %, $n = 85$; Exp1999 in 2000: 85 %, $n = 41$, in 2001: 86 %, $n = 35$; Exp2000 in 2001: 87 %, $n = 23$).

Table 8.1: Return rates of experimental and control females in 2000 and 2001. For Exp2000 females return rates are also given separately for those that raised chicks in addition to increased egg production and those that did not.

	Control	**Exp 1999**	**Exp 2000**		
				Exp 2000 *with* chicks	**Exp 2000 *without* chicks**
Return 2000	89 % (131)	93 % (46)			
Return 2001	77 % (115)	77 % (43)	83 % (42)	74 % (23)	95 % (19)

Pair bonds

The ratio of break-ups to pairs that were still breeding together in the year following increased egg production was not higher for experimental than for control pairs (ratio of break-up / together in control pairs: 0.61, n = 50; exp2000 pairs: 0.58, n = 19). However, within the experimental group, pairs that had cared for chicks were less likely to breed together in the following year than those that had not hatched a chick (tab. 8.2; logistic regression, break-up dependent on with or without chick: p = 0.018, n = 19). The break-up of a pair bond between two years can be either due to the death or non-return of one or both of the partners, or it can be caused by divorce. Divorces are defined as cases where both partners had returned but at least one was breeding with a new partner. Divorces only occurred very rarely across all treatment groups, but both divorces within the experimental group occurred amongst the pairs which had raised a chick in the experimental year (tab. 8.3).

Table 8.2: Frequency of control and Exp2000 pairs that were still breeding together in the year following increased egg removal. Includes only pairs in which both partners were colour-ringed. Exp2000 pairs are split into those that raised chicks in addition to increased egg production and those that did not.

	Control	**Exp 2000 *with* chicks**	**Exp 2000 *without* chicks**
Together	31	4	8
Break-up	19	6	1

Table 8.3: Frequency of divorces in 2001 of control and Exp2000 pairs. Includes only pairs where both partners had returned. For Exp2000 pairs divorces are split into pairs that raised chicks in addition to increased egg production and those that did not.

	Control	Exp 2000 *with* chicks	Exp 2000 *without* chicks
No divorce	30	4	8
Divorce	2	2	0

Laying dates

In the year following increased egg production, experimental females laid significantly later than control birds, despite the fact that in the experimental years the groups had not differed in their median laying dates (fig. 8.1). Two years after the manipulation Exp1999 females laid again as early as control females, and as early as they had done prior to the experiment (Friedman test of Exp1999 laying dates over a three year period: $\chi^2 = 29.2$, $p < 0.001$, $n = 28$). For Exp2000 females the magnitude of the delay in the year after the experiment was similar to the delay seen in the post-experiment season of the Exp1999 birds (Exp1999: 3 days later than controls, Exp2000: 2.5 days later). Again, individual females laid later than they had done in the previous season (Wilcoxon test of Exp2000 laying dates over two years: $Z = 3.335$, $p = 0.001$, $n = 34$). Within each year-group of experimental females there was no difference in the post-experimental laying delay for different treatments, i.e. Exp1999 fed versus un-fed, Exp2000 with versus without chicks.

Egg size

The size of first eggs laid by females in the post-experimental season was not different from first eggs produced by control females in the same year (tab. 8.4). Also, the volume of the first egg of experimental females was not smaller following increased egg production than it had been prior to it (paired t-test for egg volume in experimental and following year, Exp1999: $t_{27} = 1.384$, $p = 0.178$; Exp2000: $t_{27} = 1.867$, $p = 0.071$). There was no indication that females which bred with a new partner in the post-experimental year produced smaller eggs than they had with the old partner. As with laying dates, within experimental groups from the two years there was no difference in egg sizes for different treatments.

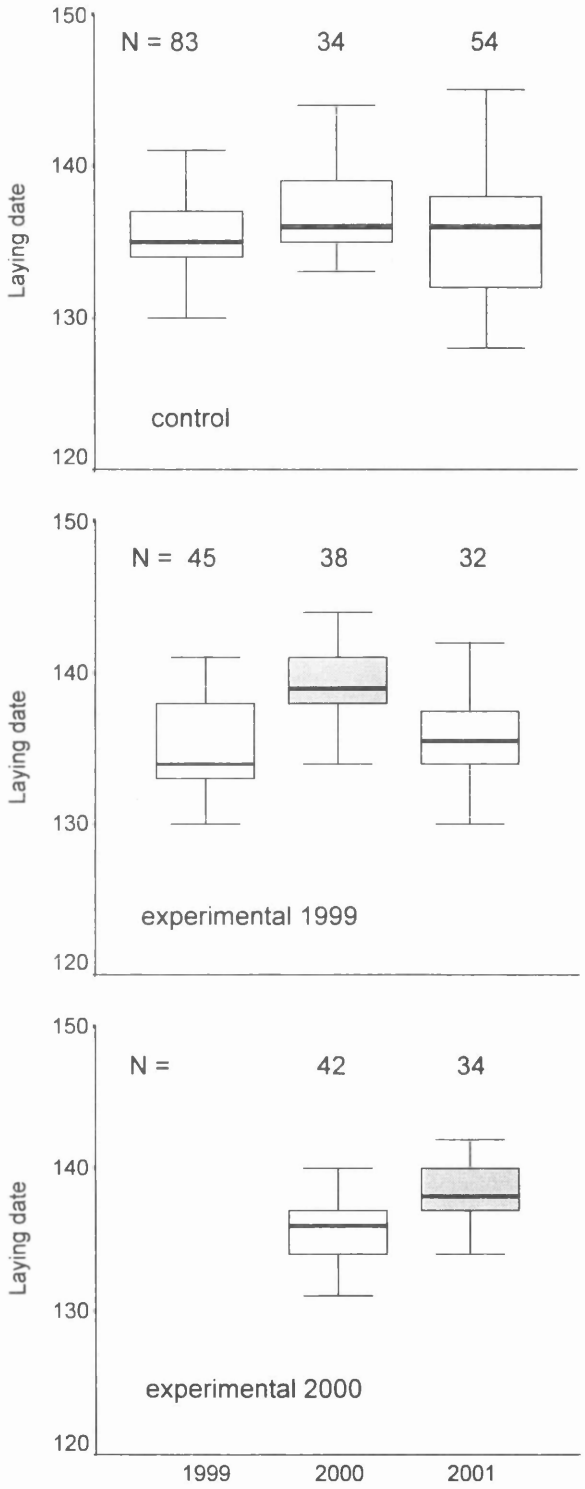

Figure 8.1: Median laying dates (Julian) of control, experimental 1999 and experimental 2000 pairs. Shaded boxes denote the years following the egg removal experiment, i.e. for experimentals 1999 laying dates in 2000, and for experimental 2000 laying dates in 2001. Median laying dates in the years following manipulation (shaded boxes) were significantly later than for all other birds in the same year (Kruskall-Wallis test for year 1999: $p = 0.431$; 2000: $p < 0.001$; 2001: $p = 0.002$).

Table 8.4: Egg volume index of first eggs from 1999 – 2001 laid by control, Exp1999 and Exp2000 females.

	Control	Exp 1999	Exp 2000
1999 1st egg	175.2 ± 11.4 (79)	171.5 ± 16.7 (45)	
2000 1st egg	173.9 ± 12.8 (26)	173.5 ± 10.6 (29)	179.1 ± 10.3 (42)
2001 1st egg	176.7 ± 12.7 (58)	172.6 ± 10.7 (32)	178.3 ± 11.5 (34)

8.5 Discussion

Inter-seasonal reproductive costs of increased egg production for female great skuas appear to be mainly paid in the form of decreased reproductive output rather than decreased adult survival. I found the same pattern of inter-seasonal costs for two different groups of females from two different experimental years. Experimentally induced production of additional eggs did not affect return rates of females nor of their partners in the following year compared to control birds. However, production of extra eggs significantly delayed breeding for females in the following year. The fact that two years post-experimentally laying dates of individual females were again as early as before the experiment, indicates that the delay was a direct consequence of the increased egg production effort. Delayed breeding is likely to have resulted in a relative decrease in reproductive output for that season, since later hatched great skua chicks tend to be in poorer body condition and survive less well during their first year than early hatched ones (Catry *et al.* 1998). Egg sizes and the probability of breeding again in the same pair combination were not affected by previously increased egg production effort.

Egg formation has been shown to be an energetically demanding process for female birds, and production of additional eggs negatively affects female condition (Bolton *et al.* 1993; Houston 1997; Veasey *et al.* 2001). The same was found in the female great skuas that were used in our experiments, and some aspects of reduced condition were still detectable a year later (chapter 3). The delay in laying dates in the post-experimental year could be caused by several mechanisms. On the one hand, delaying egg production might be a strategy of females to maintain their own body condition. They might have been able to lay earlier, but this may have incurred negative effects on their condition. On the other hand, the delay could have been forced by an inability to lay earlier due to their reduced condition or to late arrival at the colony. A third possibility is the existence of a trade-off

between laying date and egg size. Like laying date, egg size has important implications for chick performance (Furness 1983). Contrary to laying dates, egg sizes in the post-experimental year were not reduced. I am not able to quantify the likely disadvantage of a hatching delay of three days versus a reduction in hatchling size. However, these results might be an indication that the maintenance of egg size confers a higher advantage for reproductive success in great skuas than a slightly earlier laying date. It is unlikely that the delay in laying dates is due to a high number of new pair bonds amongst the experimental birds, as the probability for breeding again with the same partner was equally high in the experimental as in the control group.

Even though on average the increased egg production did not affect survival and pair bond stability, there are some noteworthy differences within the group of experimental females from the second experiment. In 2000, additionally to producing extra eggs and incubating a clutch, one group of females also cared for a chick over a varying length of time, while the other group had no chicks to look after. Females, which had cared for chicks, incurred significantly higher inter-seasonal costs than those without chicks. The probability of breeding again in the same pair bond in the year following the increased effort was significantly lower for those pairs which had cared for chicks. Whatever the reason for the break-up, it signifies a cost to the female, either because she did not return or because she has to look for a new partner. Losing the old partner has been shown to result in delayed breeding and lower fledging success (Catry *et al.* 1997). My results also indicate that the generally rare incident of divorce, i.e. both partners return but do not breed together, was higher amongst experimental pairs with chicks. Unfortunately, the sample size is not large enough to allow for a meaningful statistical analysis of divorce rates in relation to experimental treatment. Interestingly, females without chicks in 2000 had a higher return rate than any female which had cared for a chick, that is compared to the experimental as well as the control females. Contrary to extra egg production, which had a negative effect on reproduction only, raising a chick potentially incurred costs in the form of adult survival. Golet *et al.* (1998) showed in an experimental study with kittiwakes, *Rissa tridactyla*, that rearing chicks incurred survival costs as opposed to producing and incubating but not rearing chicks. Compared to the previous year, return rates of great skuas were generally lower after the 2000 season, suggesting a poor season or winter conditions. Harsh environmental conditions might have contributed to the negative effects of chick rearing on survival that year. Caring for chicks means a higher energetic investment and a longer stay at the breeding site. Both factors might contribute to the reproductive costs of chick rearing (Golet *et al.* 1998).

Compared to the two other studies that have investigated inter-seasonal reproductive costs of increased egg production, the effects found in great skuas appear relatively small (Nager *et al.* 2001; Visser & Lessells 2001). In lesser black-backed gulls, *Larus fuscus*, increased egg production affected adult survival and future reproductive output (Nager *et al.* 2001). However, all the gulls also raised chicks, which would have increased the costs. Interestingly, in gulls laying dates were not affected by the treatment, whereas egg sizes were, the exactly opposite pattern to the one I found in great skuas. This suggests that laying date and egg size are constrained by different factors in the closely related gulls and skuas. Similarly to the gulls, the reduction in future fitness in great tits, *Parus major*, incurred through extra egg production was mainly due to reduced female survival (Visser & Lessells 2001). But, as with the gulls, all treatment groups had to raise chicks.

In summary, my results show that increased egg production with or without subsequent incubation and chick rearing did incur inter-seasonal reproductive costs in the form of decreased reproductive output for females. However, these costs were higher for females which also cared for a chick during the experimental year than for those which did not. Chick rearing appears to incur costs in the form of adult survival, at least in years with relatively poor conditions.

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Chapter 9

General Discussion

9.1 Summary of main findings

Male and female great skua *Catharacta skua* chicks differed in several aspects during ontogeny. Even though the sexes were not distinguishable at hatching, by the end of the nestling period they exhibit the same size dimorphism as is found in adults (chapter 6). To reach the larger final size, females grew at a higher absolute rate and consumed more energy until the end of the nestling period (chapters 6 and 7). Overall mortality was higher for daughters, and poor hatching weight and condition had a strong effect on survival and growth of daughters but not of sons (chapters 5 and 6). These results give strong support for the size-hypothesis about sex-specific vulnerability. Hatchling weight and condition are closely related to egg size and contents, and thus reflect aspects of egg quality. Good parental condition during the nestling period could not compensate for the negative effects of poor egg quality on daughters' performance, as shown through cross-fostering (chapters 5 and 6). Egg production caused an immediate cost to the body condition of females, and with increased egg production effort the female's condition declined, as did the quality of eggs (chapters 3 and 4). With decreasing body condition and decreasing egg quality towards the end of extended laying sequences mothers produced skewed sex ratio in favour of sons (chapters 2 and 5). Considering the higher vulnerability of daughters to poor egg quality, this skew is consistent with the predictions of adaptive sex ratio adjustment. Supplementary feeding slightly improved egg quality in the extended sequences, while there was an indication that negative effects of extra egg production on female body condition were still present one year later (chapter 3 and 4). Poor body condition could explain the delayed cost of egg production in the following year, as manipulated females initiated laying significantly later (chapter 8).

9.2 What causes size-specific vulnerability?

At first sight, the higher energy demand and elevated female chick mortality in great skuas seems to fully support the size-hypothesis, stating that the larger sex is more vulnerable due to a higher energy demand and therefore more susceptible to food shortages (Clutton-Brock *et al.* 1985; Weatherhead & Teather 1991). However, my results indicate that the mechanism which causes this vulnerability might not be as direct as previously thought. Male and female great skua chicks only start to differ in size half way through the nestling period, but are not different in size, weight or condition at hatching, neither do they hatch out of differently sized eggs (chapters 2 and 6). Nonetheless, hatching condition had the strongest effect on daughters' survival and growth (chapters 5 and 6). This suggests that events during early life are responsible for immediate and later vulnerability.

Some authors suggest that in order to sustain high growth rates later (in female great skuas during the second half of the nestling period), the larger sex needs to grow larger support organs (Klaassen & Drent 1991). Increased internal growth activity is likely to increase the risk of oxidative stress because metabolic activity during growth could lead to increased free radicals (Møller *et al.* 2000). Hatching is a particularly vulnerable time for oxidative stress due to exposure to atmospheric concentration of oxygen (Surai 1999a; Surai 1999b). Mothers provision their eggs with antioxidants, such as carotenoids and vitamin E, which can inactivate free radicals and therefore prevent tissue damage (Blount *et al.* 2000). It has been suggested that this might be a physiological mechanism behind the observed negative effects later in life related to compensatory growth (Metcalfe & Monaghan 2001). In birds, the mother's ability to provide those compounds for the eggs is likely to be condition dependent (Blount *et al.* 2000; Blount *et al.* 2002). Such a condition-related difficulty of the mother in provisioning later eggs as well as early eggs could explain why daughters hatching from eggs produced when maternal condition was declining had a lower survival probability (chapter 5).

If higher energy requirements and the inability of parents to meet them were the direct cause for larger-sex vulnerability, then survival differences should be exacerbated during poor rearing conditions (Røskaft & Slagsvold 1985; Sheldon *et al.* 1998; Daunt *et al.* 2001). Reduced maternal condition has been shown to affect offspring rearing capacity (Monaghan *et al.* 1998), which might in part be caused by reduced flight performance (Veasey *et al.* 2000) and a resulting decrease in foraging efficiency. In the great skua, the decrease in female versus male chick survival probability was not steeper in a group of chicks raised by poor condition parents then when raised as singletons by good condition parents (chapter 5). This strengthens the conclusion that early negative effects are crucial. Another link between early conditions and vulnerability might exist through hormones and immune function (Potti & Merino 1996; Wikelski *et al.* 1999). In unborn lambs it has been found that males have levels of the stress hormone cortisol which are twice as high as in unborn females (D. Giussani, *pers. comm.*). A physiological side-effect of stress is a decreased immune response (Westneat & Birkhead 1998; Evans *et al.* 2000), which in turn could mediate vulnerability. However, whether the higher level of stress experienced previous to birth is a consequence of size or is specific to males is unknown.

While my results clearly support the idea that larger size negatively affects offspring performance irrespective of sex, they cannot resolve the question of the physiological mechanism behind this. However, they give a clear indication that it is less a question of total energy consumed or higher energy requirements in later life, but rather an

indirect connection with differences in early developmental physiology between the differently sized offspring. Future studies should focus on these mechanisms in order to establish the cause of size-specific vulnerability. Also, my results cannot rule out the possibility that there are detrimental effects of male-specific physiology on survival. However, the fact that the size dimorphism in the great skua is not very large but still appears to cause a considerable effect suggests that male-specific disadvantages are minor in comparison. In isolation my study could not discriminate between effects of size and heterogameity, as females are larger and also carry the different sex chromosomes. However, as pointed out in the individual chapters, jointly with the studies on sex-specific chick performance in the lesser black-backed gull *Larus fuscus* and other species with larger males, the effect of heterogameity appears negligible but cannot be excluded.

9.3 The ghost of adaptive sex ratio adjustment

The debate on whether adaptive sex ratio adjustment occurs in mammals and birds is unlikely to be solved in the near future, not to mention within one study. Despite the fact that I found survival disadvantages for daughters when hatching from eggs comparable to those in which I found a significant skew towards sons, this bias as such cannot prove adaptiveness. To conclusively show the adaptive nature of the observed skew, recruitment probability of offspring as well as future reproductive success of parents and offspring would need to be followed. Large-scale demographic data are necessary to make predictions about adaptive sex ratios and the impact of environmental changes on these predictions (Koenig & Walters 1999; Kruuk *et al.* 1999). To measure true fitness in relation to the production of different offspring sex ratios in birds has only been attempted in the long-term study of the Seychelles warbler *Acrocephalus sechellensis* by Jan Komdeur and co-workers (Komdeur *et al.* 1997; Komdeur 1998). However, my results provide evidence for the existence of a mechanism fundamental for adaptive sex ratio adjustment: facultative sex ratio manipulation. Facultative sex ratio manipulation states that mothers have the ability to vary offspring sex ratio in relation to their current situation, and that they are not physiologically restricted to produce one sex as a direct result of their condition or an invariable 50:50 ratio. Given that under very similar conditions, great skuas produced a skew to the opposite direction from gulls (Nager *et al.* 1999), and given the close relatedness of the two groups, the existence of an ability to facultatively skew the primary sex ratio appears certain. Under the paradigm of natural selection the existence of such an ability suggests a relevance to fitness. My results therefore add an important part to the growing body of evidence for sex ratio adjustment, as more studies report results of

primary sex ratios towards the predicted direction (Cordero *et al.* 2001; Albrecht & Johnson 2002).

Even though empirical evidence is growing, a main point in the debate about sex ratio adjustment is the search for a mechanism by which mammals and birds can achieve such a skew. Although fundamentally the same, the sex-determining systems of mammals and birds have one crucial difference which has strong implications for possible mechanisms. While in mammals sperm carry two different sex chromosomes and therefore determine the sex of the zygote, in birds the female gametes determine the sex (Mittwoch 1996). In domestic chickens *Gallus domesticus* the sex-determining step of meiosis occurs only a few hours before fertilisation (Sheldon 1998). In mammals, timing of fertilisation, sperm rejection or selective abortion of fertilised embryos could be mechanisms for the female to select offspring sex (Krackow 1995; Hardy 1997). The possibilities debated for birds can be split into two groups. On the one hand, female birds could detect the sex of a follicle before it is ovulated, and if it is not of the desired sex prevent ovulation and reabsorb the follicle. The process of reabsorption is known as atresia and is relatively common for small follicles in domestic chicken, but can also affect large, viable ones (Waddington *et al.* 1985). As it takes time to grow and reabsorb follicles, selective atresia should result in conspicuous laying gaps (Emlen 1997). The other possibility would be for females to influence the segregation of the sex chromosomes during meiosis (Sheldon 1998). A new analysis of the Seychelles' warbler sex ratio data gives strong evidence in favour of meiotic control (Komdeur *et al.* 2002). As birds are phylogenetically close to reptiles (Bishop & Friday 1988) factors that influence sex determination in reptiles might mitigate control in female birds. As well as temperature, hormonal levels and an interaction of both can determine offspring sex in some reptiles (Crews 1996; Bowden *et al.* 2000). Lastly, a factor that needs to be taken into account is the cost of producing a skewed sex ratio. Atresia appears more costly than influencing the outcome of meiosis, but in any case the benefits of producing a given sex ratio have to outweigh the costs of doing so. This aspect is seldom acknowledged in studies of sex ratios in birds and mammals (Oddie 1998).

9.4 Possible direct benefits to parents of sex ratio skew

As great skua daughters would be less likely to survive when hatching from an egg produced by a mother in very poor condition, they would provide a lower indirect fitness return than sons under the same circumstances. This could explain the skew produced by great skua mothers I observed (chapter 2). However, there might also be some direct

benefits to producing sons when in poor condition. I have shown that poor condition at the end of laying is unlikely to be fully recovered during the remaining breeding season (chapter 3), and can affect future reproduction (chapter 8). It is therefore desirable for the female to gain better condition as soon as possible. Even though increased egg production on its own, without caring for a chick, had a negative effect on future reproduction, this effect was even stronger for females which had reared a chick. Minimising the costs of chick rearing would be highly desirable for mothers in poor condition. As sons need less total energy to reach fledging size, the production of a son might allow the female to work less hard and spend more energy on her own recovery. In European shags *Phalacrocorax aristotelis* it has been shown that young parents, which are less efficient at foraging, are less able to raise offspring of the larger sex (Daunt *et al.* 2001). Behavioural studies of parent-offspring interactions with respect to offspring sex are needed to establish whether parents adjust their behaviour in relation to the energetic demands of differently sexed offspring (Lessells *et al.* 1998). Following on from this thought is the question about the impact that raising one sex has compared to raising the other on survival and future reproduction of the parents.

In my study, I concentrated on the effects of reproduction on the female parent. As parental care in the great skua is shared between the sexes and pair bonds usually last for many years, the question arises how increased effort into egg production, reduced female condition and offspring sex affect future reproductive success of the male partner. Does the male compensate for the poor condition of the female and increase his effort to aid condition recovery? In the lesser black-backed gull for example, during incubation males seem to compensate fully for reduced condition of females (Jones 2001). As pair bond is very important for breeding success in the great skua, it would be expected that males try to increase their effort to allow females to recover.

9.5 Closing Remarks

In sexually size-dimorphic species, the larger sex needs more energy to reach fledgling size and generally shows a higher rate of intrinsic mortality, regardless of the direction of size dimorphism. High vulnerability appears to be a physiological effect of the developmental pattern of the larger sex, even though not necessarily directly connected to higher energy demand *per se*. Thus, reproductive value of the two sexes can differ according to environmental circumstances. Mothers seem to have the ability to facultatively skew the primary offspring sex ratio. Whether observed sex ratios are

generally adaptive remains to be proven, but clearly the adaptiveness of a given sex ratio can be conferred through advantages to the parents as well as to the chicks.

9.6 References

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